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Veterinary Therapeutics

Research in Applied Veterinary Medicine™

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Apparent ELISA Detection Times for Albuterol After Administration with the Torpex™ Equine Inhaler Device*

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ABSTRACT

Single doses of one, three, and six actuations (120 µg albuterol/actuation) and multiple daily doses (six actuations per dose four times daily) for 5 days of aerosol albuterol sulfate were sequentially administered to each of six horses using an equine inhaler device (Torpex™, Boehringer Ingleheim Vetmedica, Inc.). A 2-week washout period was allowed between each dose. ELISA testing revealed no evidence of albuterol in urine at 24 hours after any single-dose administration. Results indicated that 48 hours or longer should be allowed for albuterol to be cleared from urine after single doses. When given at the maximum recommended rate of six actuations per dose four

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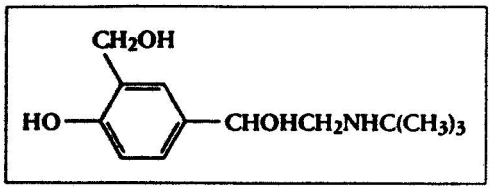


Figure 1. *Structure of albuterol.*

times a day for 5 days, urine samples tested by ELISA showed no evidence of albuterol at 48 hours after the final dose. Testing of nasal swabs by ELISA demonstrated the presence of albuterol for 8 hours after each single dose, and some horses might have detectable levels of albuterol in nasal swabs for several days following administration of multiple doses. As a guideline for withdrawal time, 72 hours or longer should be allowed after administration of aerosol albuterol sulfate to horses before participation in equestrian competitions that are regulated for detection of certain performance-enhancing substances. However, these recommendations were based on a small sample of horses and the specific ELISA test used and interpreted as described. Factors specific to individual horses may influence these detection times.

■ INTRODUCTION

Albuterol is a relatively selective β-2 adrenoreceptor agonist (Figure 1) used as a bronchodilator in the treatment of human asthma and chronic obstructive pulmonary disease.[1] Aerosolized albuterol sulfate administered by a prototype Torpex™ (Boehringer Ingelheim Vetmedica, Inc.) equine inhalation device (Figure 2) at 360 and ~720 µg, was an effective bronchodilator in horses with recurrent airway obstruction without causing unpleasant side effects.[2] Onset of action with albuterol is rapid (5 minutes), and its effects can last from 30 minutes to 3 hours.[3] Unlike formoterol and salmeterol, which are lipophilic and have long durations of action, albuterol is hydrophilic and exhibits a rapid onset and short duration of action.[4]

The novel Torpex™ equine inhalation device effectively and efficiently administers aerosolized medications in a synchronized manner from a metered-dose inhaler into the respiratory tract. Because the lung is the target organ for albuterol, administration by this device allows efficient delivery of aerosol albuterol sulfate to the sites of action in the respiratory tract. The efficiency of the inhaler delivery system, therefore, serves to reduce the dose of aerosol albuterol sulfate required to attain a therapeutic response. This reduction in the required dose is expected to bring the added benefit of reducing both incidence and intensity of adverse responses and, in the case of competition horses, may reduce the possibility of inadvertent medication identifications.

Classified by the Association of Racing Commissioners International, Inc. (ARCI) as a Class 3 (i.e., drugs that may or may not have generally accepted medical use in race horses, but the pharmacology of which suggests less potential to affect performance than drugs in Class 2) bronchodilator, albuterol may potentially alter the athletic performance of horses, particularly if the animal has bronchospasm and bronchoconstriction associated with reversible airway obstruction.[4,5] Therefore, its detection in samples collected following a horse's performance could lead to significant sanctions against the trainer. In competition horses, the detection of high-potency medications, such as albuterol, is largely dependent on ELISA testing. Aerosol albuterol sulfate was administered to six Thoroughbred horses at different recommended doses using the Torpex™ equine inhaler for drug delivery, and ELISA tests were used to detect albuterol and its metabolites in serum, urine, and nasal swabs from these horses.

MATERIALS AND METHODS

Horses

Six mature Thoroughbred mares weighing 413 to 602 kg each were used. Horses were kept in a 20-acre field until they were placed in box stalls and provided with water and hay ad libitum. All horses were acclimated to their stalls 24 hours prior to participation in the study. The animals were maintained on grass hay, and concentrate feed (mixture of oats and an alfalfa-based protein pellet) was fed twice a day. Horses were vaccinated annually for tetanus and dewormed every 3 months with a commercial ivermectin product. A routine clinical examination was performed prior to each of the four stages of the study. The horses were managed according to the rules and regulations of the University of Kentucky's Institutional Animal Care and Use Committee, which approved the experimental protocol.

Treatments

Four dose rates for aerosol albuterol sulfate were administered and evaluated in a series, and all six horses were treated with the same dose of aerosol albuterol sulfate on a given treatment day. All dosing was accomplished with the Torpex™ equine inhaler provided by the manufacturer. On each day of treatment, horses each received a single dose of one (120 µg albuterol/actuation), three, or six actuations, or six actuations four times per day at 6-hour intervals for 5 days (the maximum recommended duration of aerosol albuterol sulfate administration using the commercial inhaler). A two-week washout period was allowed for each horse before administering the next sequential dose.

Sample Collection

A venous blood sample was collected into a serum-separator tube from each horse, according to standard postrace testing practice in Kentucky. Complete urine collection was accomplished with a Foley catheter at 0, 1, 2, 4, 6, and 8 hours after administration, and a Harris flush tube (24 Fr × 152.4 cm) was used for this purpose at 24, 48, 72, 96, 120, 144, and 192 hours after administration. Urine samples were stored at −20°C until assayed. Nasal swabs were collected by inserting a 40-cm (16-inch) cotton nasal swab into the ventral nasal meatus of the horse up to approximately 15 cm. A pretreatment nasal swab sample was taken from each horse; however, this procedure was difficult to accomplish several times daily from the same horse, so a different horse was sampled by nasal swabs at each evaluation (one horse per time) after treatment with each dose.

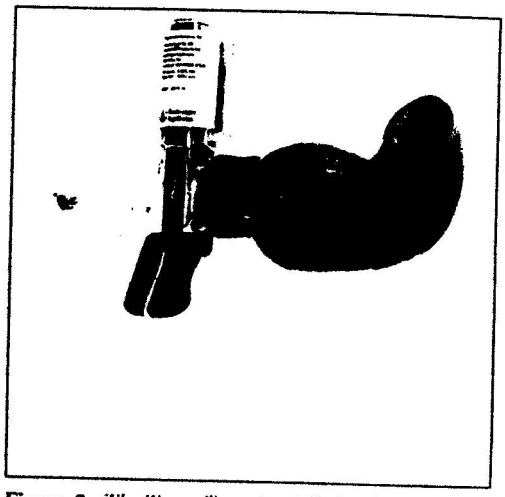


Figure 2. *The Torpex™ equine inhaler device used for delivery of aerosol albuterol sulfate.*

ELISA Methods

ELISA tests were performed using the Neogen® Bronchodilator Group ELISA kits (Neogen Corporation). ELISA buffer (0.1 M potassium phosphate-buffered saline, pH 7.4, with 0.1% bovine serum albumin), wash buffer (0.01 dM phosphate buffer, pH 7.4, with 0.05% Tween® 20), specific terbutaline-

horseradish peroxidase (drug–enzyme) conjugate solution, and K-Blue Substrate® (3, 3', 5, 5' tetramethylbenzidine with hydrogen peroxide; Neogen Corporation) were obtained as part of the ELISA kits.

Authentic albuterol standard (salbutamol, Sigma-Aldrich) was prepared at 1 mg/ml in methanol and diluted to appropriate concentrations in assay buffer (0.1 M potassium phosphate-buffered saline, pH 7.4 with 0.1% bovine serum albumin).

The one-step ELISA tests were performed as previously described[7] and according to the manufacturer's instructions, with albuterol standard curves (0.1 to 10 ng/ml) in assay buffer, blank-serum matrix, or blank-urine matrix. To create the serum or urine matrix (blank serum or urine collected from each horse before dosing) standard curves, the assay was started by adding 20 µl of standard, test, or control samples to the appropriate wells in duplicate. To each well containing the samples, 180 µl of diluted (1:180) drug–enzyme conjugate was added. To each well containing the standards in buffer, 20 µl of blank serum or urine and 160 µl of diluted (1:160) drug–enzyme conjugate were added. The plates were then placed on a microplate shaker briefly, covered, and incubated at room temperature for 45 minutes.

For nasal swab samples, 5 ml of assay buffer was added to each sample in the vial with extraction by vigorous mixing by vortex. The nasal swab samples were assayed using a buffer standard curve. The assay was started in the same manner as for serum and urine (20 µl of standard, test, or control samples added to the appropriate wells in duplicate, followed by 180 µl of diluted [1:180] drug–enzyme conjugate).

After incubation of the samples, the wells were inverted, and any remaining liquid was removed by tapping the plate on a lint-free towel. Each well was washed with 300 µl of diluted wash buffer (0.01 dM phosphate buffer, pH 7.4 with 0.05% Tween® 20) three times. The plates were inverted and tapped dry between each washing. K-Blue Substrate® (150 µl) was added to each well, and the plates were mixed by gentle shaking on a microplate shaker for 30 minutes. The optical density (OD) of each well was read at a wavelength of 650 nm with an automated microplate reader. Samples with OD less than that of the highest standard were diluted appropriately with assay buffer and reassayed. Because ELISA testing does not specifically identify albuterol, its metabolites, or structurally related substances, all ELISA quantifications were identified as "albuterol equivalents."

Statistical Analyses

Estimated concentrations of albuterol equivalents were calculated for the serum samples using the serum matrix standard curve, for the urine samples using the urine matrix standard curve, and for the nasal swab samples using the buffer standard curve. The data were plotted as % maximum activity (OD at 650 nm) versus time, or as albuterol equivalent concentration (ng/ml) versus time. Where appropriate, statistical analysis was performed by applying the central limit theorem to a sampling distribution of the sample mean in a *t*-test distribution, with the single assumption that the low sample number is representative of the population in a binomial distribution.[8]

■ RESULTS

The ELISA kit used in this study readily detects various β-2 adrenoreceptor agonists and antagonists with varying sensitivities depending on the sample matrix and the compound being tested, as shown in Table 1. In this table, the I-50 value (the drug concentration that inhibits the ELISA reaction 50%) represents the relative detection sensitivity for each individual

TABLE 1. Sensitivities for Various β-2 Adrenoreceptor Agonists and Antagonist (Propranolol) in Different Matrices in the Neogen® Bronchodilator Group ELISA Test Kit

	Sensitivity (I-50; ng/ml in Given Matrix)*					
Compound	*EIA† Buffer*	*Porcine Urine*	*Canine Urine*	*Equine Urine*	*Equine Plasma*	*Equine Serum*
Terbutaline	0.5	0.8	1.0	1.1	0.6	0.9
Clenbuterol	1.2	1.3	1.4	1.1	2.6	1.2
Salbutamol/Albuterol	1.5	2.4	2.2	2.7	3.0	1.1
Pirbuterol	1.5	5.0	3.0	7.0	3.0	4.0
Metaproterenol	2.7	7.5	6.1	6.5	9.0	5.0
Propranolol	15.0	40.0	20.0	30.0	45.0	80.0

*I-50 = drug concentration that inhibits the ELISA reaction 50%.
†EIA = Equine infectious anemia.

compound. The ELISA was highly sensitive for terbutaline, followed by clenbuterol, albuterol, and metaproterenol, respectively. The calculated cross-reactivity of the ELISA with various bronchodilator agents is shown in Table 2. Cross-reactivity refers to the ELISA antibody-binding of a compound compared with that of the original compound for which antibody was developed (in this case, terbutaline 100%). The cross-reactivity of the ELISA used in this study was as follows: clenbuterol 45%, albuterol 35%, pirbuterol 33%, metaproterenol 20%, and propranolol 3.3%. None of the other compounds listed showed significant cross-reactivity. The standard curves of the various bronchodilator agents in ELISA buffer are presented in Figure 3. Additionally, ELISA standard curves for albuterol in various biological matrices shown in Figure 4 indicate the most sensitive assay for albuterol was in equine serum and assay buffer.

Analysis of serum samples after treatments revealed relatively little inhibition of ELISA response. For all time points, the maximum activity after treatment averaged less than 25% inhibition and was thus not considered substantially different from the baseline value of the pretreatment sample (Figures 5 and 6).

Inhibition of ELISA activity for urine samples following single administrations of albuterol is shown in Figure 7. These data indicate that at least 24 hours should be allowed after intranasal administration of six or fewer actuations for the urine to eliminate albuterol.

TABLE 2. Cross-reactivity of Neogen® Bronchodilator ELISA Group Kits with Various Agents*

Compound	*ELISA Cross-reactivity (%)†*
Terbutaline	100
Clenbuterol	45
Salbutamol/Albuterol	35
Pirbuterol	33
Metaproterenol	20
Propranolol	3.3
Isoproterenol	0.98
Colterol	0.45
Metoprolol	0.10

*Cross-reactivity = antibody binding to a compound other than the original compound for which antibody was developed.
†% cross-reactivity = (I-50 [drug concentration that inhibits the ELISA reaction 50%] of the original analyte/I-50 of the cross-reacting compound) × 100.

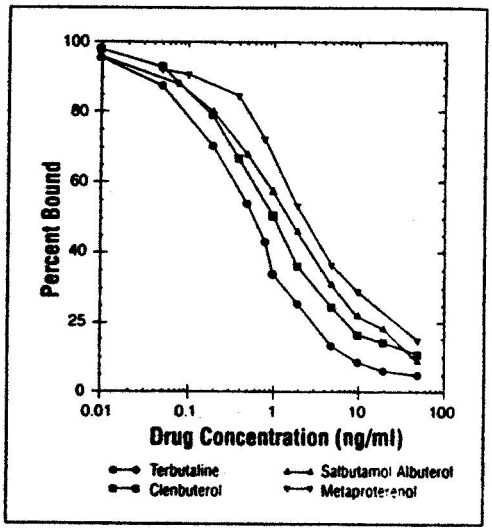


Figure 3. *Standard curves for various β-2 agonist bronchodilator agents in ELISA buffer.*

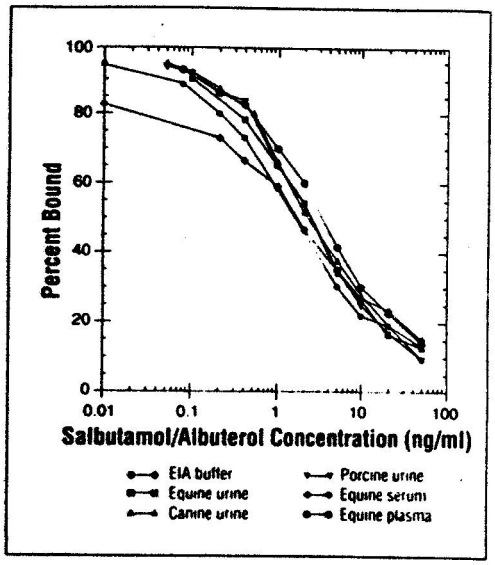
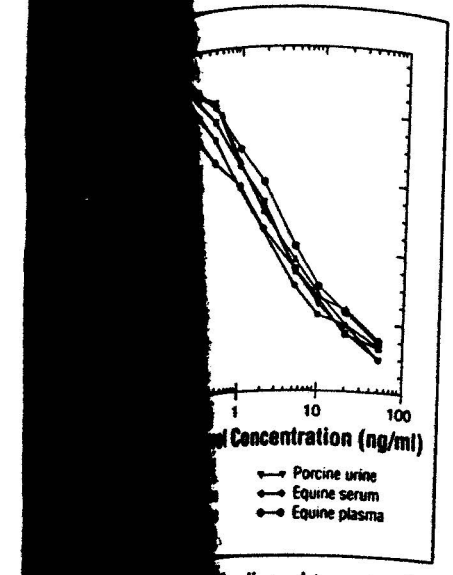


Figure 4. *Standard curves for albuterol in various biological matrices using the Neogen® Bronchodilator Group ELISA kits. The most sensitive assays for albuterol were obtained in equine serum and ELISA buffer.*

The apparent peak concentrations of albuterol equivalents in urine samples were dose-related ($r^2 = 0.999$); (Figure 8). The mean peak urinary concentrations of albuterol equivalents occurred within 2 to 4 hours after administration and were 1.02 (one actuation), 4.89 (three actuations), and 10.15 (six actuations) ng/ml. The mean peak urinary concentrations of albuterol equivalents were 0.8 (one actuation), 2.68 (three actuations), and 6.22 (six actuations) ng/ml at 8 hours after dosing, and the urine samples did not contain ELISA detectable levels of albuterol equivalents at 48 hours after administration.

The urinary inhibition of the ELISA tests following administration of multiple doses of albuterol for 5 days is presented in Figure 9. Mean urinary concentrations of albuterol equivalents following this multiple-dosing regimen are shown in Figure 10. The mean peak urinary concentrations of albuterol equivalents ranged from 15 to 17.5 ng/ml during the 5 days that treatments were given and declined rapidly after the last administration. Concentrations of albuterol equivalents were 10.95 ng/ml at 8 hours after the last dose (Figure 10). During the dosing and for at least 8 hours after the last dose, all urine samples gave strong positive indications of the presence of albuterol. None of the urine samples had detectable concentrations of albuterol equivalents at 24 hours after the last dose.

The percent inhibition of the ELISA test in nasal swab samples following increasing doses of aerosol albuterol sulfate administered by Torpex™ is shown in Figure 11. The data suggest that nasal swabs up to at least 6 hours after treatment can give a positive ELISA response after a single dose of either three or six actuations. The apparent concentrations of albuterol equivalents in the nasal swab extracts following single administrations at one, three, or six actuations are shown in Figure 12. Peak concentrations of albuterol equivalents were obtained within 1 to 2 hours post-dosing and



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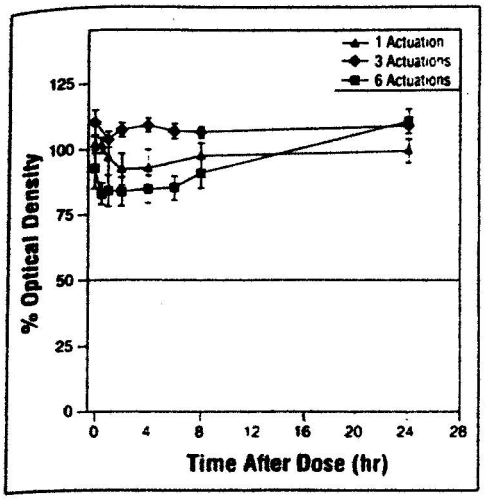


Figure 5. *Mean optical density (OD) of albuterol (± standard error of the mean) detected by ELISA in serum samples from six horses given a single treatment (one, three, or six actuations per treatment) of aerosol albuterol sulfate using the Torpex™ equine inhaler device. The horizontal line represents 50% inhibition of the maximum OD.*

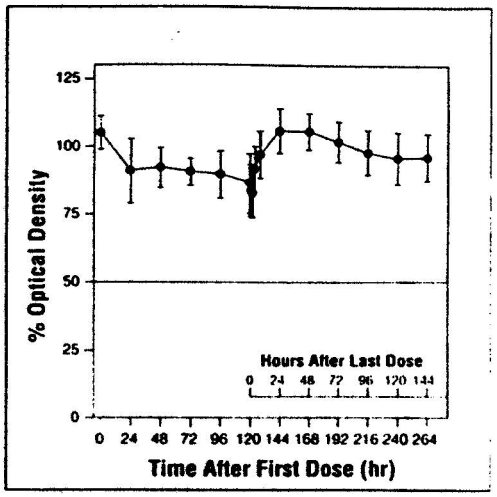


Figure 6. *Mean optical density (OD) of albuterol (± standard error of the mean) detected by ELISA from serum samples of three of six horses treated with aerosol albuterol sulfate (six actuations [120 µg albuterol/actuation] per treatment) using the Torpex™ equine inhaler device four times daily at 6-hour intervals for 5 days. The horizontal line represents 50% inhibition of the maximum OD.*

were 6.76 (one actuation), 34 (three actuations), and 210 (six actuations) ng/ml.

ELISA inhibition produced by nasal swabs following the multiple-dosing regimen is shown in Figure 13. These data suggest that nasal swabs collected up to at least 24 hours after the last dose may have detectable levels of albuterol equivalents by ELISA. The peak concentrations of albuterol equivalents in nasal swab samples taken on Day 5 (day of last treatment) ranged from 11 ng/ml to 90 ng/ml (Figure 14). The mean estimated concentration of albuterol equivalents from these nasal swab samples was 40 ng/ml at Day 5 and 0.13 ng/ml at 120 hours after the initial dose (24 hours after the final dose) (Figure 14).

■ DISCUSSION

Albuterol is a relatively selective β-2 adrenergic receptor agonist, and each actuation of the Torpex™ device delivers 120 µg of albuterol sulfate. As a bronchodilator, albuterol has the potential to alter the athletic performance of horses,[5] particularly if the animal experiences bronchospasm and bronchoconstriction associated with reversible airway bronchoconstriction.[5,6] Because albuterol is an ARCI class 3 agent, its detection in postperformance samples may lead to sanctions against the trainer or other people connected with the horse.

The major therapeutic advantage of the inhaler device used to deliver aerosol albuterol sulfate is that the system delivers active compound directly and deeply into the lung resulting in rapid onset of therapeutic action (5 to 15 minutes). Administration by this route also bypasses the gastrointestinal tract and liver metabolism, either of which can significantly reduce or alter the bioavailability of an orally administered drug. Administration of aerosol albuterol sulfate with this inhaler device can

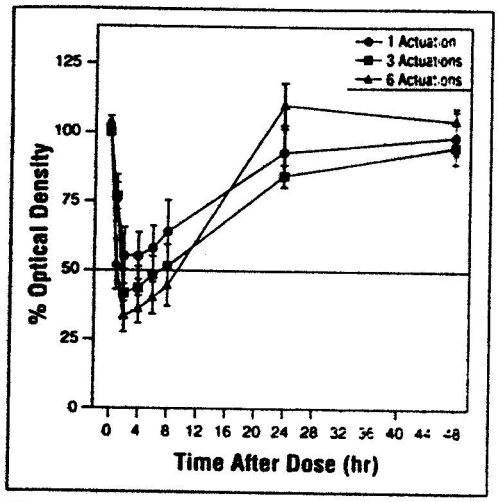


Figure 7. *Mean optical density (OD) of albuterol (± standard error of the mean) detected by ELISA in urine samples from six horses given a single treatment (one [n = 5], three, or six actuations per treatment) of aerosol albuterol sulfate using the Torpex™ equine inhaler device. The horizontal line represents 50% inhibition of the maximum OD.*

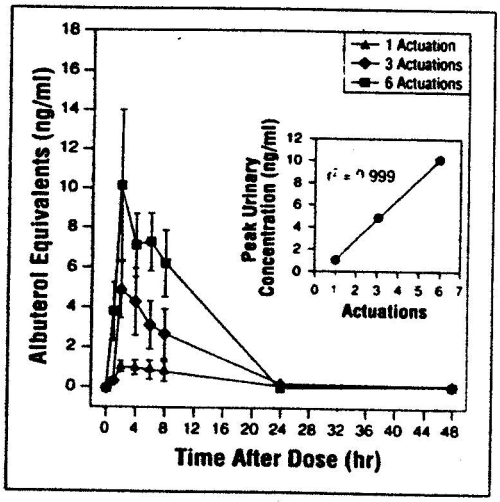


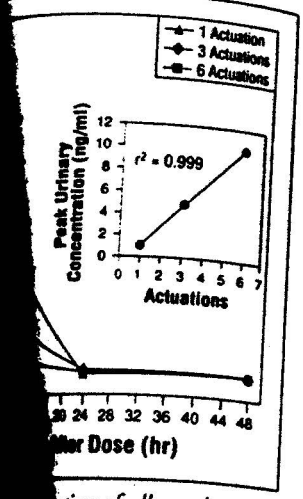
Figure 8. *Mean concentrations of albuterol equivalents (± standard error of the mean) detected by ELISA in urine samples from groups of five or six horses given a single treatment (one, three, or six actuations per treatment) of aerosol albuterol sulfate using the Torpex™ equine inhaler device and correlation of the mean peak urinary concentrations and doses of aerosol albuterol sulfate.*

significantly reduce the dose required for therapeutic efficacy. These characteristics of the inhaler device provide high therapeutic efficacy and a low incidence of side effects.

The goal of this study was to provide guidelines for veterinarians and regulators regarding the period of detection by ELISA for drug and metabolite residues in biologic fluids of horses when aerosol albuterol sulfate is given by the Torpex™ inhaler. Regulatory control of albuterol generally involves ELISA screening of postrace urine samples, followed by mass spectral confirmation of its presence. ELISA tests were performed in this study to detect active drug and metabolites in serum, urine, and nasal swab samples following administration of aerosol albuterol sulfate by the Torpex™ inhaler.

Application of the ELISA test in this study showed very little ELISA response in any of the posttreatment serum samples tested. For all time points, the maximum inhibition of ELISA activity after treatment was not substantially different from baseline values. In the absence of unusual extraction procedures, the ELISA test used in this study is unlikely to detect albuterol in serum samples after administration of these doses by the equine inhaler device.

Conversely, albuterol equivalents were readily detected in urine samples following administration of various recommended doses of aerosol albuterol sulfate. The mean peak urinary concentrations of albuterol equivalents occurred within 2 to 4 hours after treatment, and the majority of the urine samples did not contain ELISA-detectable concentrations of albuterol equivalents at 24 hours after administration. To be conservative, however, it would be prudent to allow at least an additional 24 hours (total of 48 hours or longer) for albuterol or its metabolites to clear after single adminis-



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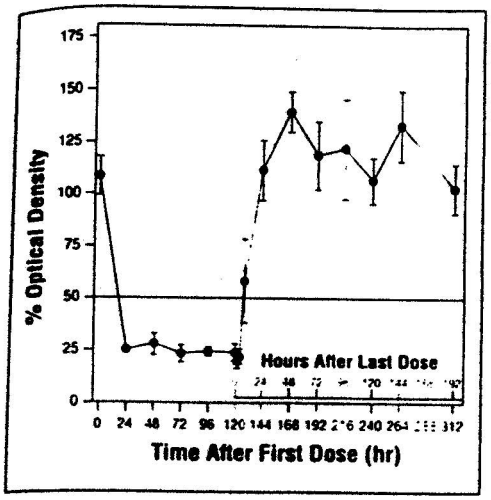


Figure 9. *Mean optical density (OD) of albuterol (± standard error of the mean) detected by ELISA in urine samples from six horses each given four treatments (six actuations per treatment) per day of aerosol albuterol sulfate at 6-hour intervals for 5 days using the Torpex™ equine inhaler device. The horizontal line represents 50% inhibition of the maximum OD.*

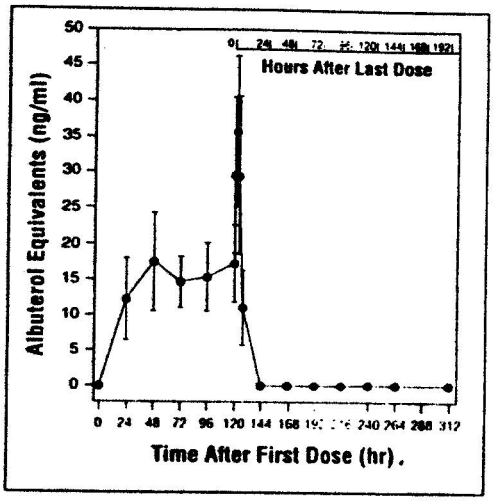


Figure 10. *Mean concentrations of albuterol equivalents (± standard error of the mean) detected by ELISA in urine samples from six horses each given four treatments (six actuations per treatment) per day of aerosol albuterol sulfate at 6-hour intervals for 5 days using the Torpex™ equine inhaler device.*

trations. The maximum recommended duration of treatment with aerosol albuterol sulfate is four daily doses (six actuations per dose) for 5 days. Following administration of the maximum recommended dose in the present study, the mean peak urinary concentrations of albuterol equivalents ranged between 15 and 17.5 ng/ml. After multiple dosing for 5 days, the mean urinary concentrations of albuterol declined rapidly, and none of the urine samples had a detectable level of albuterol equivalents 24 hours after the last dose. However, because the present study involved a small sample of horses and might not be representative of a larger population, an additional 48 hours (total of 72 hours), or longer is recommended for albuterol or its metabolites to be cleared from the urine.

ELISA analysis of nasal swab material revealed that peak concentrations of albuterol equivalents were obtained within 1 to 2 hours after administration of a single dose (one, three, or six actuations per dose). Although the data used to determine elimination of aerosol albuterol sulfate were based on a single horse per dose at each evaluation time, it is likely that nasal swabs collected up to 6 hours or longer after administration of three or six actuations per day for one day will still yield a positive ELISA response. The data also suggest that any nasal swab collected 24 hours or longer after the last dose of the multiple treatment regimen may have detectable levels of albuterol equivalents by ELISA.

■ CONCLUSION

There was no evidence of albuterol in the urine detected by ELISA testing at 24 hours after administration of a single daily dose of aerosol albuterol sulfate by the Torpex™ equine

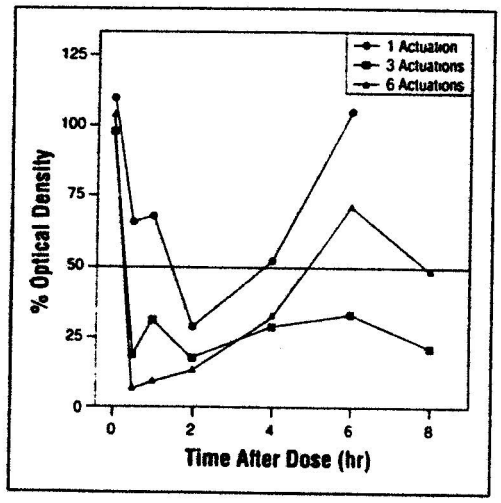


Figure 11. *Mean optical density (OD) of albuterol (± standard error of the mean) detected by ELISA in nasal swabs from six horses given a single treatment (one, three, or six actuations per treatment) of aerosol albuterol sulfate using the Torpex™ equine inhaler device. The horizontal line represents 50% inhibition of the maximum OD.*

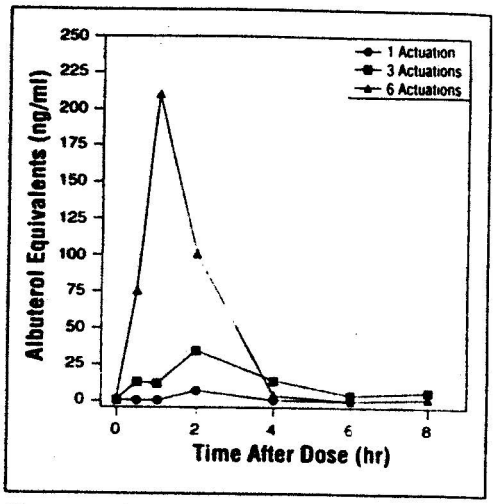
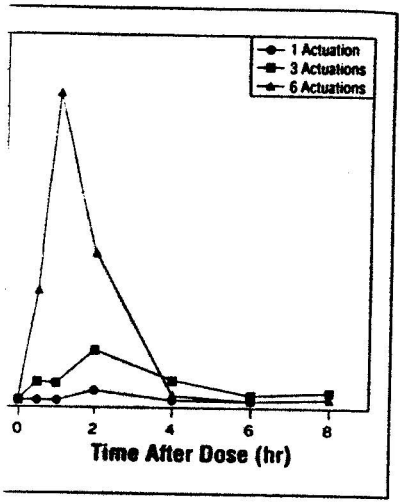


Figure 12. *Mean concentrations of albuterol equivalents detected by ELISA in nasal swab samples from six horses each given a single treatment (one, three, or six actuations per treatment) of aerosol albuterol sulfate using the Torpex™ equine inhaler device.*

inhaler at one, three, or six actuations per dose (120 µg/actuation). However, 48 hours or longer is recommended as a conservative period for albuterol to be eliminated from the urine following administration of these single daily doses before entering a horse into a competition that is regulated by drug testing.

Nasal samples had detectable levels of albuterol for 8 hours after administration of multiple doses of aerosol albuterol sulfate in the Torpex™ inhaler; it is possible that individual nasal samples could inhibit the ELISA test for several days after the final dose.

The results suggest that it is very unlikely that screening of serum samples by the ELISA test described will detect albuterol in equine serum, urine, or nasal swab samples 72 hours or longer after administration of aerosol albuterol sulfate by the Torpex™ inhaler at the reported doses. In interpreting and applying these findings, it must be kept in mind that these results are guidelines only and were developed in a relatively small sample of horses. The findings are based on the sensitivity of the ELISA test used as described, and this test differs somewhat in sensitivity from the one used at racetracks. Modification of this test or its interpretation to increase its sensitivity has the potential to increase the probability of detection of albuterol. Conversely, a jurisdictional threshold for albuterol, such as 1 ng/ml, which is the "decision level" in California, would serve to decrease the probability of detection. Beyond this, factors specific to individual horses may also influence detection times. As always, prior to administration of any medication to a horse entering an event that is likely to test for medications or drugs, it is advisable to consult your veterinarian or other experts concerning any unique regulatory or testing characteristics related to the medication, the horse, or the event in question.



. Mean concentrations of albuterol equiva-ed by ELISA in nasal swab samples from six given a single treatment (one, three, or six per treatment) of aerosol albuterol sulfate orpex® equine inhaler device.

ılts are guidelines only and were de-n a relatively small sample of horses. ngs are based on the sensitivity of the st used as described, and this test dif-what in sensitivity from the one used cks. Modification of this test or its ıtion to increase its sensitivity has the to increase the probability of detec-buterol. Conversely, a jurisdictional for albuterol, such as 1 ng/ml, the "decision level" in California, ve to decrease the probability of de-beyond this, factors specific to indi-ırses may also influence detection always, prior to administration of cation to a horse entering an event ely to test for medications or drugs, able to consult your veterinarian or ırts concerning any unique regulato-ng characteristics related to the med-ıe horse, or the event in question.

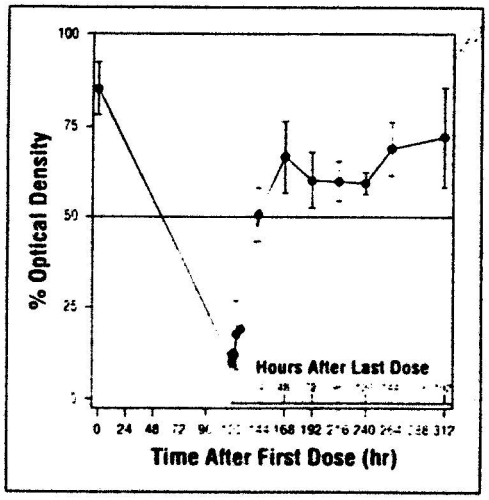


Figure 13. *Mean optical density (OD) of albuterol (± standard error of the mean) detected by ELISA in nasal swab samples from five of six horses each given four treatments (six actuations per treatment per day of aerosol albuterol sulfate at 6-hour intervals for 5 days using the Torpex® equine inhaler device. The horizontal line represents 50% inhibition of the maximum OD.*

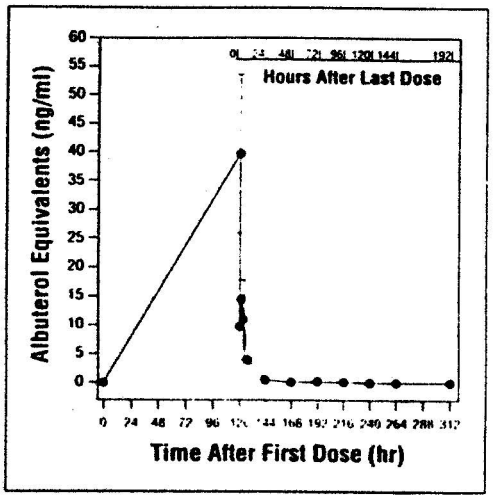


Figure 14. *Mean concentrations of albuterol equivalents (± standard error of the mean) detected by ELISA in nasal swab samples from five of six horses given four treatments (six actuations per treatment) per day of aerosol albuterol sulfate at 6-hour intervals for 5 days using the Torpex® equine inhaler device.*

REFERENCES

1. Torneke K, Ingvast Larsson C, Appelgren H: A comparison between clenbuterol, salbutamol and terbutaline in relation to receptor binding and in vitro relaxation of equine tracheal muscle. *J Vet Pharmacol Ther* 21(5):388–392, 1998.
2. Nava S, Karakurt S, Rampulla C, Braschi A, Fanfulla F: Salbutamol delivery during non-invasive mechanical ventilation in patients with chronic obstructive pulmonary disease: a randomized, controlled study. *Intensive Care Med* 27(10):1627–1635, 2001.
3. Derksen FJ, Olszewski MA, Robinson NE, et al: Aerosolized albuterol sulfate used as a bronchodilator in horses with recurrent airway obstruction. *Am J Vet Res* 60(6):689–693, 1999.
4. Lofdahl J: Pharmacological similarities and differences between beta-2 agonists. *Respir Med* 95(suppl B):S7–S11, 2001.
5. Bailey J, Colahan P, Kubilis P, Pablo L: Effect of inhaled beta (2) adrenoreceptor agonist, albuterol sulphate, on performance of horses. *Equine Vet J Suppl* 30:575–580, 1999.
6. Sasse HL, Hajer R: NAB 365, a beta-2 receptor sympathomimetic agent: Clinical experience in horses with lung disease. *J Vet Pharmacol Ther* 1:241–244, 1978.
7. Stanley SD, Gairola CG, Diana JN, et al: Development and characterization of an ELISA for cotinine in biological fluids. *Inhal Toxicol* 5:403–413, 1993.
8. Daniels WW: *Biostatistics: A Foundation for Analysis in the Health Sciences*. New York: John Wiley & Sons; 1983:94–121.