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Determination of salbutamol in human plasma and urine by gas chromatography–mass spectrometry

T. SRICHANA, G. P. MARTIN, A. KICMAN*, C. WALKER* AND C. MARRIOTT

*Department of Pharmacy, King's College London, Manresa Road, London SW3 6LX, and *Drug Control Centre, King's College London, Manresa Road, London SW3 6LX*

Salbutamol remains the first line treatment for many bronchospastic diseases. Following inhalation administration, the concentration of salbutamol in plasma and urine is small and previous published methodology was found to lack the required assay sensitivity (Hindle and Chrystyn, 1992, Clark *et al.*, 1996).

The aim of this study was to develop a highly specific and sensitive analytical method for the determination of the drug in plasma and urine concentration. The accuracy, precision and drug recovery of the extraction method was investigated.

Solid phase extraction was carried out using silica (130 mg) cartridges (Bond Elut™, Varian, USA). The cartridges were conditioned with 2 ml of methanol, followed by 2 ml of water. Urine samples (1 ml) containing salbutamol 10-50 ng or plasma (3 ml) containing salbutamol 1-10 ng were prepared and mixed with 0.5 ml terbutaline (100 ng ml^{-1} in water) as the internal standard. Each sample was loaded onto the cartridge and washed with 2 ml of water, followed by 1 ml of dichloromethane, 0.2 ml of ethyl acetate and finally 2 ml of acetonitrile. The cartridge was then dried for 5 min by allowing air to flow through using a Vac Elut system (Varian, USA), before finally recovering the salbutamol and internal standard by eluting with 1 ml of 2% (v/v) triethylamine in methanol. The final sample eluant was dried under a stream of nitrogen. The dried extracts were further dried in a vacuum dessicator over phosphorous pentoxide for 1 h before derivatising by adding 50 µl N-methyl-N-(tri-methylsilyl) trifluoroacetamide (MSTFA) and warming at 60°C for 15 min to form the trimethylsilyl (TMS) derivatives of salbutamol and terbutaline. A 1 µl aliquot was analysed using gas chromatography (Hewlett Packard 5890 series II plus, USA) by direct injection onto a capillary column (25 m length * 0.2 mm i.d.) coated with crosslinked poly-dimethylsiloxane attached to a mass selective detector (HP 5970A), using helium gas at 0.6 ml min^{-1} as a carrier. The injector and detector were maintained at 250°C whilst the temperature of the oven was initially set at 100 °C for 3 min and then increased at 15 °C min^{-1} up to 300 °C and held at 300 °C for 5 min. The mass spectrometer was operated using electron impact mode. Analysis was accomplished by selected ion monitoring at m/z 356 and 86 for tri-TMS-terbutaline and m/z 369 and 86 for tri-TMS-salbutamol. The choice of qualifier ions was determined from the full scan spectra of the derivatised compounds.

Extraction was shown to be satisfactory since there was no interference from either the plasma or urine sample in the regions of peak interest. The retention time was 10.97 min for derivatised terbutaline and 11.47 min for derivatised salbutamol. Calibration curves were constructed by calculating peak height ratio of derivatised salbutamol relative to the derivatised terbutaline (y) at each known concentration (x, ng ml^{-1}). Linear regression was performed and the curves were found to be linear over the specified concentration ranges for plasma ($y = 0.041x-0.005$, $r^2 = 0.995$, 1-10 ng ml^{-1}) and urine ($y = 0.044x-0.015$, $r^2 = 0.999$, 10-50 ng ml^{-1}). The limit of detection in plasma was 0.25 ng ml^{-1}. The results of validation are shown in the Table.

Table: Accuracy, precision and recovery of salbutamol extracted from plasma and urine samples (n = 6, mean ± sd), P = Plasma, U = Urine

	Conc (ng ml^{-1})	Detected conc(ng ml^{-1})	Precision (% CV)+	% recovery
P	1	0.96 ± 0.02	4.51	95.7
	5	4.83 ± 0.17	3.25	97.4
	10	9.74 ± 0.17	1.85	98.7
U	10	9.88 ± 0.22	2.21	100.9
	30	29.48 ± 0.37	1.39	98.8
	50	50.19 ± 0.42	1.02	104.7

+ % CV- percent coefficient of variation

In conclusion, a specific GC-MS procedure has been developed for the determination of salbutamol in plasma and should have sufficient sensitivity to determine the pharmacokinetic profile of salbutamol following administration of the drug by means of dry powder inhalers.

Clark, D.J., Gordon-Smith, J., McPhate, G., and Lipworth, B. J. (1996), Thorax, 51, 325-326.

Hindle, M. and Chrystyn, H. (1992), Br. J. Clin. Pharm., 34, 311-315.