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The determination in human plasma of 1-hydroxy-2-naphthoic acid following administration of salmeterol xinafoate

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Abstract: The clinical development of salmeterol xinafoate, the 1-hydroxy-2-naphthoic acid salt of salmeterol, a potent long acting β_2 agonist bronchodilator, has required the development of a method for the determination of 1-hydroxy-2-naphthoic acid (HNA), in human plasma. A sensitive, accurate and precise method was, therefore, required to enable the pharmacokinetic profile to be established. HNA was determined in human plasma using a semi-automated procedure with solid-phase extraction using an automated analytical sample processor (AASP) and high-performance liquid chromatography (HPLC) with fluorescence detection. The method was sensitive to 10 ng ml^{-1}. The method is specific for HNA with respect to endogenous plasma components and has been shown to be robust, accurate and precise. Over four independent assay runs, the relative standard deviations (RSD) of the quality control samples (QC) were 1.6, 2.4 and 5.5% at 180, 100 and 40 ng ml^{-1}, respectively. A pharmacokinetic profile of HNA in man has been established from a single dose kinetic study in healthy volunteers following an oral dose of 500 μg salmeterol xinafoate, equivalent to 225 μg HNA. Maximum plasma concentrations attained at 1 h after dosing ranged between 35.3 and 66.8 ng ml^{-1} and were within the calibration range of the assay.

Keywords: *HPLC; solid-phase extraction; plasma; 1-hydroxy-2-naphthoic acid; salmeterol xinafoate.*

Introduction

β_2-Adrenoceptor agonist drugs are important in the treatment of reversible obstructive airways disease. Until recently, available drugs were short acting and, therefore, of limited efficacy in the treatment of nocturnal asthma. SEREVENT™, salmeterol xinafoate, has been developed as a potent β_2-adrenoceptor agonist having the desired pharmacological profile of a long acting bronchodilator [1, 2]. The prolonged duration of action may, therefore, be advantageous over existing therapy.

Salmeterol xinafoate is the 1-hydroxy-2-naphthoic acid (HNA) salt of salmeterol. There is little information available on the disposition of HNA; consequently the acid was treated as a xenobiotic substance for purposes of drug development. Since this entailed analysis of samples from animal and clinical studies, a sensitive, accurate and precise method was required. A semi-automated solid-phase extraction method with high-performance liquid chromatography (HPLC) with fluorescence detection was developed with a limit of quantitation (LOQ) of 10 ng ml^{-1}. The method was validated according to Shah *et al.* [3] and used for the determination of HNA in samples arising from clinical pharmacokinetic studies. An example of a profile from these studies is presented in this report.

Experimental

Chemicals, standards and materials

HNA was obtained from Aldrich Chemicals (Gillingham, UK). HPLC grade methanol was obtained from Rathburn Chemicals Ltd (Walkerburn, UK). Hydrochloric acid (Analar), sodium hydroxide (Analar), di-sodium hydrogen orthophosphate dihydrate (Analar), potassium dihydrogen orthophosphate (Analar) and orthophosphoric acid (85%, Analar) were all obtained from BDH Chemicals Ltd (Poole, UK).

Instrumentation

The HPLC system comprised of a Kratos Spectraflow 400 solvent delivery system (Anachem, Luton, UK), and an automated

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analytical sample preparation (AASP) system (Varian Associates, Walnut Creek, CA, USA). The AASP C_{18} sample preparation cartridges, 10 in each cassette, were supplied by Jones Chromatography (Hengoed, Wales, UK). Separation was achieved using a Spherisorb ODS II cartridge column (5 μm, 100 × 4.6 mm i.d.), with a guard column (10 × 4.6 mm i.d.) packed with the same material. The guard column was replaced after approximately 250 samples to prolong the life of the analytical column. The column temperature was maintained using a model 7961 HPLC column block heater (Jones Chromatography). The fluorescence detector was a Hitachi F1000 (BDH Ltd, Dagenham, UK). Data handling was carried out with a Trilab Chromatography Data system (Trivector Systems International, Sandy, UK).

Preparation of standards

Calibration standards at 10, 20, 50, 100, 150 and 200 ng ml^{-1} were prepared by mixing control human plasma with aqueous solutions of HNA. The calibration standards in plasma were prepared either prior to each assay run, or in a batch to be stored frozen (−20°C) until required. HNA was found to be stable in control plasma for 12 months when stored at −20°C.

Sample preparation

Samples and standards were prepared for analysis by solid-phase extraction using the AASP prep-station. The C_{18} cartridges were washed sequentially with methanol (1 ml), distilled water (0.9 ml) and phosphate buffer (pH 2.0, 0.05 M, 1 ml). Distilled water (1.3 ml) was added to the cartridge reservoir followed by the plasma sample (150 μl) and hydrochloric acid (10%, 150 μl). The acidified plasma solution was eluted through the cartridge and the extract was washed with phosphate buffer (pH 2.0, 0.05 M, 1 ml). The cassette was then transferred to the AASP module for HPLC analysis.

Chromatographic analysis

Chromatographic analysis was carried out at 40°C using an ODS-II spherisorb column and guard column with a mobile phase of phosphate buffer (0.05 M adjusted to pH 5.0 with orthophosphoric acid) and methanol in the ratio 6:4 with a flow rate of 1 ml min^{-1}. Detection was by fluorescence at excitation wavelength of 310 nm and emission wavelength of 410 nm.

Quantification

The calibration standards were analysed in duplicate and interspersed with the samples throughout the assay run. The assays were controlled by the analysis of QC samples at three concentrations assayed in duplicate during each analysis. Peak areas were recorded and calibration lines were constructed and HNA concentrations in the QC and study samples were quantified by comparison with these lines. Samples containing HNA at concentrations outside the calibration ranges were diluted with control plasma to within the calibration range.

Method validation

Validation was carried out to determine the accuracy, precision and specificity of the method. The intra-assay accuracy and precision were determined by carrying out six replicate analyses of plasma samples spiked at the concentration of the calibration standards. The inter-assay accuracy and precision were studied by assaying in duplicate QC samples at three concentrations in four independent assay runs.

The extraction efficiency was determined by comparing the mean peak areas of HNA obtained from replicate analyses of plasma extracts, at each calibration standard concentration, with the areas obtained from aqueous solutions at the same concentrations. The specificity of the method was investigated for clinical studies by assaying plasma samples from six human volunteers.

Applications

This method has been established to determine the levels of HNA in human plasma samples taken during clinical studies to establish the pharmacokinetic profile of HNA in healthy volunteers following ethical approval.

Six healthy male volunteers received a single oral dose of 500 μg salmeterol xinafoate, equivalent to 225 μg HNA. Blood samples were taken before and at the following time points after dosing: 0.5, 1, 2, 4, 6, 8, 12 and 24 h and then twice weekly for the next 5 weeks. Plasma was separated by centrifugation and was assayed for HNA. The results were

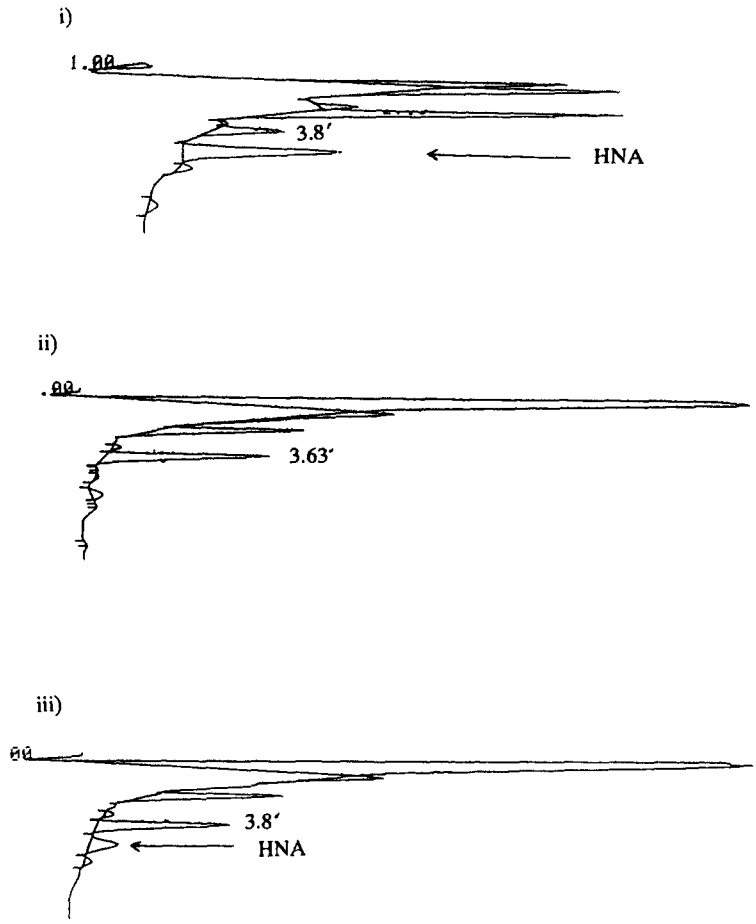

Figure 1
Chromatograms resulting from analysis of (i) calibration sample spiked at 100 ng ml^{-1}, (ii) predose, and (iii) 2 h post dose sample from healthy volunteer following a single oral dose of the equivalent of 500 μg HNA.

used to determine the apparent pharmacokinetics in man.

Results and Discussion

Examples of typical chromatograms of a calibration sample and a volunteer are shown in Fig. 1. The retention time of HNA was 4.4 min and was consistent during the analytical runs. Calibration lines were approximately linear, though throughout the analyses, a quadratic regression was found to give a better fit to the data suggesting that there was some loss of linearity over the calibration range in some assays.

The mean recovery at each concentration over the calibration range is given in Table 1. The means of the extraction recovery over the concentration range of 50–200 ng ml^{-1} were between 71.4 and 77.3%, respectively. Higher efficiencies were observed at 20 ng ml^{-1} with 88.6% and with 112% at 10 ng ml^{-1}.

Table 1
Recovery of 1-hydroxy-2-naphthoic acid (HNA) from plasma for the range 10–200 ng HNA per ml

Nominal HNA concentration (ng ml^{-1})	% Recovery		Mean (%)
10	100.5	123.4	112
20	85	91.4	88.6
50	70.3	72.4	71.4
100	72.4	72.5	72.5
150	72.5	74.6	73.5
200	76.1	78.5	77.3
Overall mean			8.25
RSD (%)			19
n			6

The intra-assay variation determined at each calibration point over the range 10–200 ng ml^{-1} is reported in Table 2. The relative standard deviation (RSD) at 20 ng ml^{-1} was 8% and was 2.1% at 200 ng ml^{-1}. The variation at the 10 ng ml^{-1} level was somewhat higher at 19.2%. The bias (% error) was

Table 2
Intra-assay variation and bias for the determination of 1-hydroxy-2-naphthoic acid in plasma for the range 10–200 ng ml^{-1}

Nominal concentration of HNA (ng ml^{-1})	Mean concentration (ng ml^{-1})	Number of replicates (n)	RSD (%)*	Bias† % error
10	11.0	6	19.2	+10.2
20	19.4	6	8.0	−3.0
50	45.6	6	6.6	−8.7
100	96.0	6	2.6	−4.9
150	143	6	2.2	−4.7
200	189	6	2.1	−5.6

* Relative standard deviation (RSD) $= \frac{\text{standard deviation}}{\text{mean concentration}} \times 100.$

† % Error $= \frac{\text{mean concentration} - \text{nominal concentration}}{\text{nominal concentration}} \times 100.$

Table 3
Inter-assay variation and bias for the determination of 1-hydroxy-2-naphthoic acid in plasma for the range 10–200 ng ml^{-1}

Nominal concentration of HNA (ng ml^{-1})	Mean concentration (ng ml^{-1})	Number of replicates (n)	RSD (%)*	Bias† % error
40	38.8	8	5.5	−3.0
100	101	8	2.4	+0.6
180	173	8	1.6	−4.0

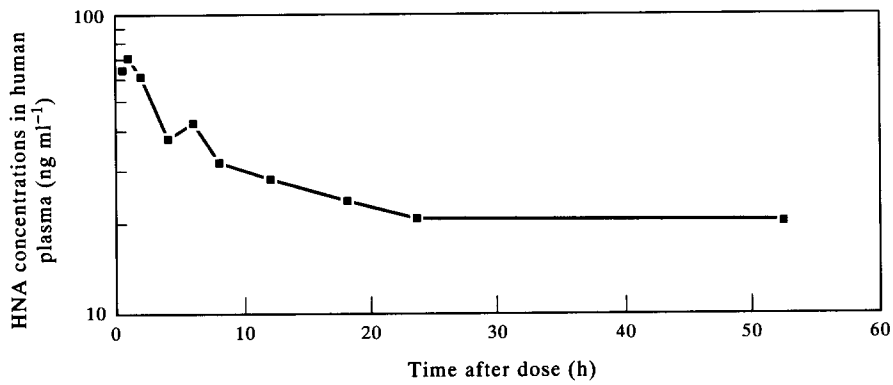

Figure 2
Typical pharmacokinetic profile from a healthy volunteer following an oral dose of salmeterol xinafoate, equivalent to 225 μg HNA.

+10.3% at 10 ng ml^{-1} and between −3.0 and −8.7% over the remaining range. The range observed for the 10 and 20 ng ml^{-1} concentrations of 85–123% may have been due to endogenous interference in the HPLC assay, effective at the lower concentrations. This too was reflected in the intra-assay variation with a RSD of 19.2% at 10 ng ml^{-1}. This was still suitable for the analysis of human samples.

The results from the analysis of QC samples from four independent runs were used to calculate the inter-assay precision and bias and are reported in Table 3. The RSD values at the three concentrations, 40, 100 and 180 ng ml^{-1} were 1.6, 2.4 and 5.5%, respectively. The bias at 180 ng ml^{-1} was −4.0% with +0.6 and −3.0% for 100 and 40 ng ml^{-1}, respectively.

The degree of accuracy, precision and sensitivity obtained with the method was satisfactory and did not necessitate the use of an internal standard. The method has been shown to be robust in sustained use for up to 12 months, successfully assaying batches of up to 80 samples in addition to the calibration and QC samples.

The method was used to determine levels of

HNA in samples taken during volunteer studies to determine the pharmacokinetics. An example of a typical profile is presented in Fig. 2. Following an oral dose of 500 μg salmeterol xinafoate, the maximum plasma concentrations of HNA were attained at 1 h after dosing and ranged between 35.3 and 66.8 ng ml^{-1}.

In conclusion, the method described is rapid, sensitive, accurate and precise. It is suitable for use in support of pharmacokinetic studies and for therapeutic monitoring of plasma concentrations of HNA.

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