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Determination of loratadine and pheniramine from human serum by gas chromatography–mass spectrometry

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Abstract

In this work, a method for the determination of the antihistaminic drugs loratadine and pheniramine from human serum is presented. Serum samples are extracted under basic conditions with hexane–*n*-amyl alcohol (95:5, v/v), the analytes are reextracted into diluted hydrochloric acid and, after basification, are once again extracted into the organic phase. The samples are measured by GC–MS. The limits of detection of the assay are 0.5 ng/ml for loratadine and 2 ng/ml for pheniramine. The R.S.D.s in the day-to-day precision test for loratadine are 7.0% at 20 ng/ml and 12.4% at 2 ng/ml. For pheniramine, the R.S.D. are 6.4% at 300 ng/ml and 10.2% at 20 ng/ml.

1. Introduction

Loratadine and pheniramine are two antihistaminic drugs of common use in the treatment of allergic diseases.

For the determination of loratadine, a RIA method [1] and more recently an HPLC procedure [2] and a GC procedure applying a nitrogen–phosphorus detector [3] are known from the literature. For pheniramine, a GC–alkali flame ionization detector procedure [4], an HPLC procedure using post-column derivatisation with chemiluminescence detection [5] and a GC–MS method especially fitted for screening purposes [6] are described.

We here present a procedure for the determination of the two substances from human serum that is suitable to attend clinical and scientific trials as well as forensic analysis in cases of misuse of the drugs. For both substances, the preparation of the samples is identical and the determination is, due to the applied GC–MS procedure, very specific and sensitive and the dynamic range of quantitation is very wide.

2. Experimental

2.1. Apparatus

A gas chromatograph HP 5890 Series II plus (Hewlett Packard, Waldbronn, Germany) with an electronic pressure programmer, split–splitless injector and HP 7673 autosampler is used. The analytes are separated on a HP-Ultra 1 capillary column (25 m × 0.200 mm I.D.). The detection is carried out on a HP 5972 MSD mass-selective ion detector (all Hewlett Pac-

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kard). Data are collected and analyzed by a Hewlett Packard DOS ChemStation, software version C.01.05.

2.2. Chemicals

Loratadine (batch 92-6-0428), pheniraminehydrogenmaleate (batch E330) and etoloxaminehydrochloride (batch 940483) are kind gifts of Essex Pharma (Munich, Germany), Hoechst (Frankfurt, Germany) and Deutsche Hydrierwerke (Rodleben, Germany), respectively. Methanol and *n*-hexane are purchased from Baker (Gross-Gerau, Germany; quality grade: Baker analyzed), *n*-amyl alcohol p.a. grade is from Merck (Darmstadt, Germany). Helium, quality 5.6, is purchased from Messer-Grießheim (Magdeburg, Germany). Pooled human serum is a gift from the Institut für Transfusionsmedizin und Immunhämatologie mit Blutbank (University Hospital Magdeburg, Germany).

2.3. Sample collection

The blood samples (about 10 ml) are collected into glass tubes. To separate serum from blood cells, the samples are centrifuged for 10 min at 2400 *g*. After centrifugation, the serum samples are frozen at −20°C until analysis.

2.4. Sample preparation

To 1 ml serum, 0.1 ml of a solution of the internal standard (I.S.) etoloxamine in water (1 μg/ml; i.e., 1.13 μg/ml etoloxaminehydrochloride) and 0.1 ml of 1 *M* NaOH in water are added. This mixture is extracted with 3 ml of 5% *n*-amyl alcohol in *n*-hexane by shaking for 35 min. After phase separation, the organic phase is transferred into a tube containing 3 ml 1 *M* hydrochloric acid and is reextracted by shaking for 20 min. The remaining organic phase is discarded and the aqueous phase is basified by adding 0.5 ml 10 *M* NaOH. This mixture is extracted again by shaking with 1 ml of 5% *n*-amyl alcohol in *n*-hexane. Finally, the organic phase is evaporated, the residue reconstituted in 50 μl *n*-hexane, transferred into autosampler vials and forwarded for GC–MS analysis.

2.5. Calibration and quality control samples

A stock solution of pheniraminehydrogenmaleate is made by dissolving 29.6 mg (= 20 mg of free base) in 100 ml water. For the free base loratadine, 5 mg are dissolved in 25 ml methanol. The pheniramine solution is diluted by a factor of 10 and drug-free human serum is spiked with this solution to yield calibration samples in the concentration range of 20 to 300 ng/ml. The loratadine solution is diluted by a factor of 100 and drug-free human serum is spiked to yield calibration samples in the concentration range of 2 to 20 ng/ml.

Quality control samples are prepared in the concentrations 20 and 300 ng/ml for pheniramine and 2 and 20 ng/ml for loratadine, respectively. Before and after each sample block, a set of these quality control samples is measured.

2.6. Chromatographic conditions

Carrier gas for the chromatographic separation is helium at a flow-rate of 1 ml/min. This flow-rate is held constant over the run time of the temperature program. The temperature program starts at 100°C, constant for 2 min. By measuring loratadine, the temperature is raised with 15°C/min up to 310°C and held constant for 2.5 min. The appropriate program for the pheniramine assay raises the temperature with 10°C/min up to 250°C, constant for 1 min. In both cases, the temperatures of the injector and the MS-interface are 270°C and 300°C, respectively. An amount of 1 μl of the sample is injected in the splitless mode and the split valve is opened after 2 min with a split flow of 15 ml/min.

Under these conditions, the retention time of pheniramine is 12.6 min and for loratadine 17.2 min. The I.S. etoloxamine elutes in the loratadine assay at 11.9 min and in the pheniramine assay at 15.3 min. For pheniramine, the ions m/z 58.3 and m/z 169.2 are observed. Etoloxamine is monitored at m/z 86.2 and m/z 283.2 and loratadine at m/z 382.1

3. Results and discussion

3.1. Chromatography and mass spectrometry

In Fig. 1, the electron impact mass spectra obtained at an ionization energy of 70 eV are depicted.

For loratadine, the base peak at m/z 382 corresponds to the molecular ion (M^+). The main fragmentation pattern results from cleavages through the pyrimidine ring of the molecule, e.g. m/z 266 corresponds to M^+ minus the fragment EtNHC(O)OEt. The molecular ion of pheniramine at m/z 240 can hardly be seen in the mass spectrum. The main fragments correspond to the cleavage at the tertiary carbon atom connecting the two aromatic rings, from which the fragment ion m/z 169 arises, and the α-

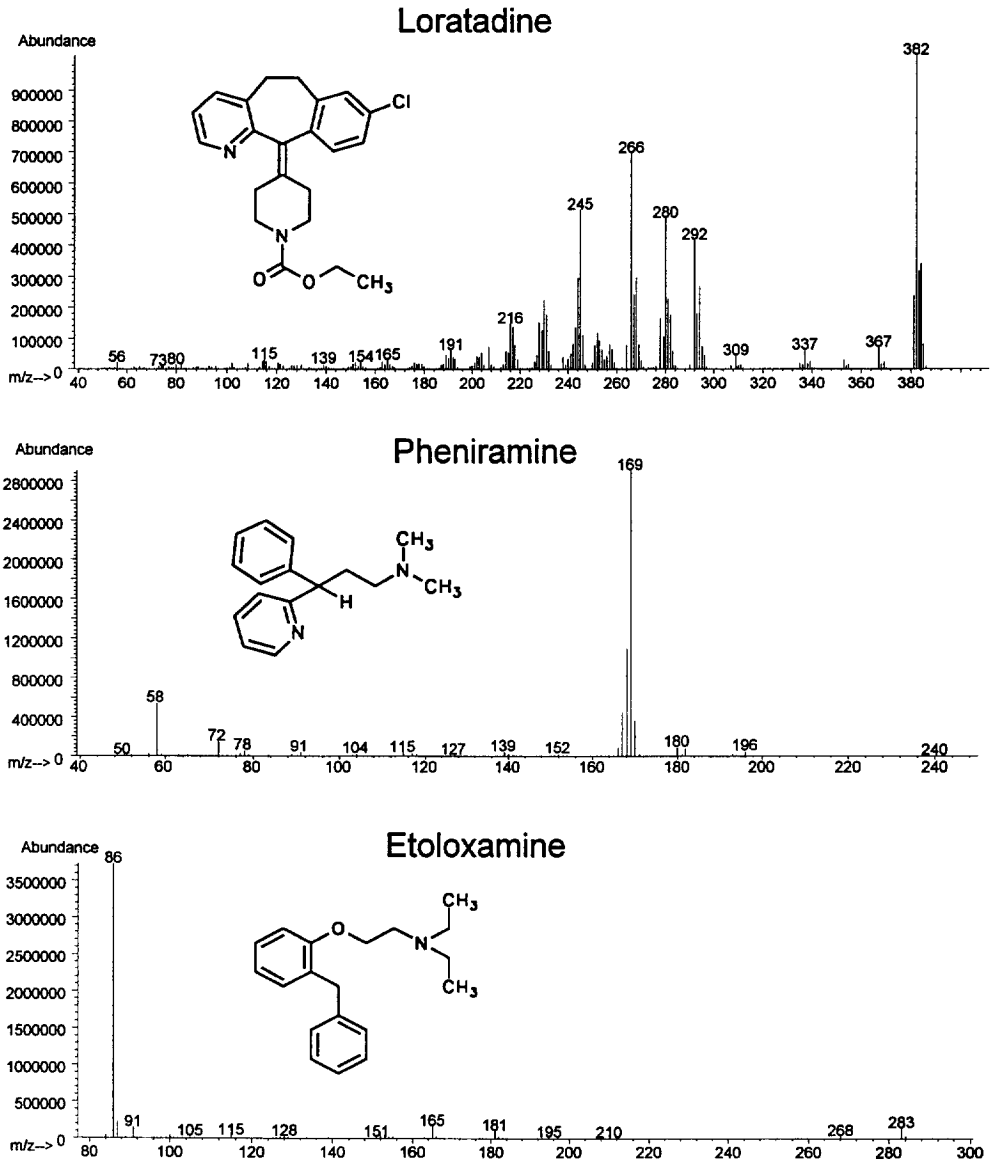

Fig. 1. 70 eV electron impact mass spectra and molecular structures of loratadine, pheniramine and the I.S. etoloxamine.

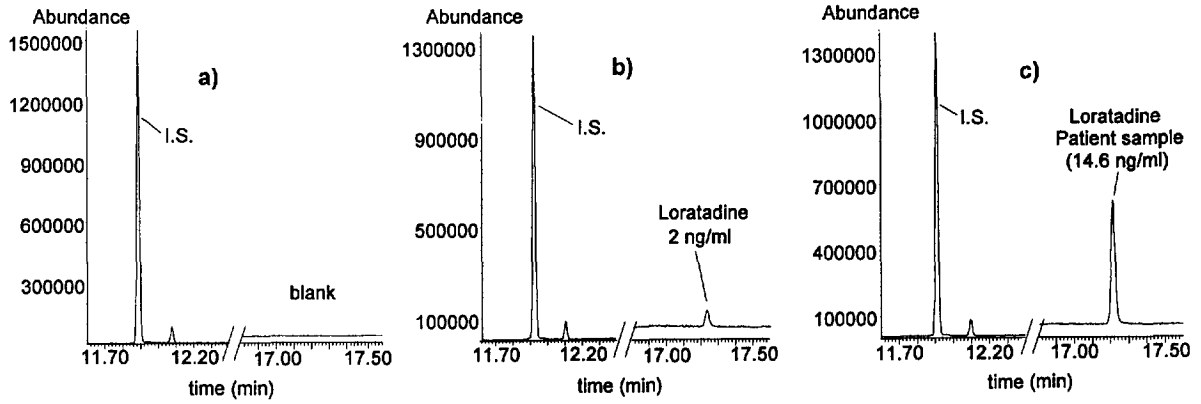

Fig. 2. Chromatograms obtained with the loratadine assay: (a) drug-free human serum extracted; (b) serum spiked with 2 ng/ml loratadine; (c) patient serum containing 14.6 ng/ml loratadine. For the sections of the chromatograms from 16.9 min to 17.6 min, the abundance axis is multiplied with a factor of 100.

cleavage, from which the fragment ion $(CH_2\text{-}N(CH_3)_2)^+$ with m/z 58 results. The analogous α-cleavage product $(CH_2\text{-}N(CH_2\text{-}CH_3)_2)^+$ produces the base peak of the mass spectrum of the I.S. etoloxamine. The molecular ion with m/z 283 only reaches an intensity of 5% of the base peak. From these mass spectra the M^+ at m/z 382, which is both intense and selective, is chosen for quantitation of loratadine. For pheniramine, the fragment ion at m/z 169 serves for quantitation and the ion at m/z 58 supplies an additional qualification of the chromatographic peak. In the same way, the fragment ion at m/z 86 is used for the quantitation of etoloxamine, and the less intense but more selective fragment ion at m/z 283 is used for qualification.

Fig. 2 shows some chromatograms from the loratadine assay and Fig. 3 the corresponding chromatograms from the pheniramine assay. In both cases, chromatogram (a) represents the yields from blank human serum, (b) from serum spiked with a concentration in the range of the limit of quantitation, and (c) from a patient's sample, containing one of the drugs.

As it can be seen, no endogenous substances interfere with the loratadine assay; hence the limit of detection is given from the sensitivity of the detector. In the case of pheniramine, some

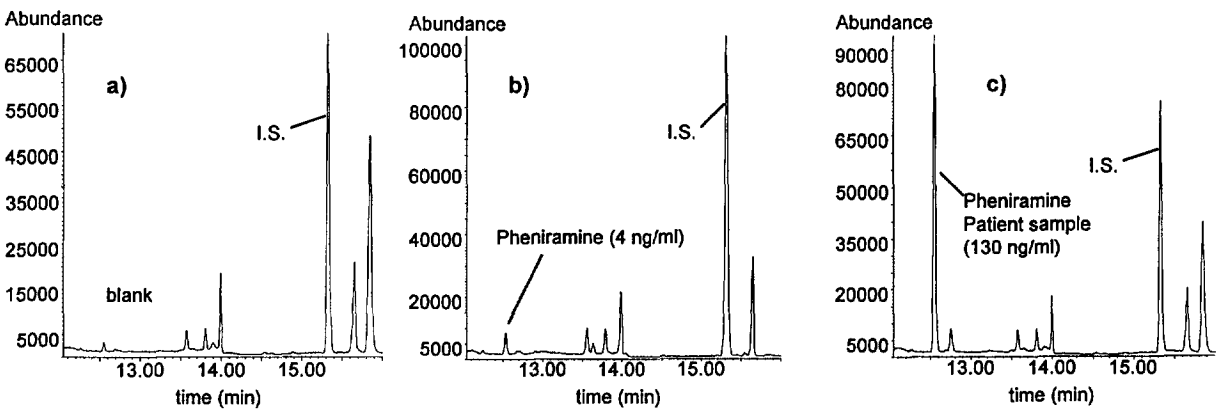

Fig. 3. Chromatograms obtained with the pheniramine assay: (a) drug-free human serum extracted; (b) serum spiked with 4 ng/ml pheniramine; (c) patient serum containing 130 ng/ml pheniramine.

small peaks from endogenous substances elute at times near the pheniramine peak. To maximize the resolution in this area, the pheniramine temperature program (see above) is applied. If pheniramine samples are run with the loratadine temperature program, then pheniramine elutes at 10.2 min, but due to the interferences, the limits of detection and quantitation are slightly higher.

3.2. Extraction procedure

The somewhat complicated extraction procedure is necessary to obtain extracts free from interfering substances. The extraction yield for both substances and the I.S. is about 70% of the theoretical value, while the losses are mainly due to incomplete phase separations. The I.S. etoloxamine is an old antihistaminic drug with only little use in therapy nowadays. It shows a close structural relation to pheniramine. In comparison to loratadine, it possesses the same essential moieties like an aliphatic tertiary amine and two aromatic rings. Since it exhibits extraction yields similar to those of the analytes, it can be used to control the losses via the extraction procedure. Therefore, the quantitation is not impaired by incomplete extraction yields.

3.3. Concentration–response relationship

For loratadine, the calibration function in the range of 2 to 20 ng/ml has the parameters intercept = $1.1790 \cdot 10^{-4}$ (S.D. = $1.609 \cdot 10^{-4}$) and slope = $5.342 \cdot 10^{-4}$ (S.D. = $1.296 \cdot 10^{-5}$); the regression coefficient has a value of 0.9977. The calibration function for pheniramine has, in the range of 4 to 300 ng/ml, the following parameters: intercept = $5.81 \cdot 10^{-2}$ (S.D. = $3.75 \cdot 10^{-2}$), slope = $6.15 \cdot 10^{-3}$ (S.D. = $2.28 \cdot 10^{-4}$) and the regression coefficient $r = 0.9946$.

The linearity of the calibration functions is proven up to 60 ng/ml for loratadine and up to 600 ng/ml for pheniramine. Hence, without modification of the assay, high concentration samples can be quantified by simply expanding the calibration range.

3.4. Limit of detection and quantitation

The limit of detection is defined as three times the noise level of a blank chromatogram. The resulting limits of detection are 0.5 ng/ml for loratadine and 2 ng/ml for pheniramine. The limit of quantitation is defined as three times the value of the limit of detection. The resulting values are 1.5 ng/ml for loratadine and 6 ng/ml for pheniramine.

3.5. Precision and accuracy

In Table 1, the results of the day-to-day and the intra-day precision tests are summarized. None of the tests exceeds the limit of 15% R.S.D., which is the highest acceptable value in our laboratory.

The day-to-day precision test has been performed with the quality control samples. The mean values of the results are very close to the spike levels (maximum difference = 5%). Hence, the accuracy of the assays is proved.

Table 1
Day-to-day and intra-day precision

Compound	Day-to-day ($n = 10$)		Intra-day ($n = 10$)	
	Mean (ng/ml)	R.S.D. (%)	Mean (ng/ml)	R.S.D. (%)
Pheniramine	18.1	8.4	20.6	10.2
	261	8.3	305	6.4.
Loratadine	2.0	4.7	2.1	12.4
	23.4	5.8	19.8	7.0

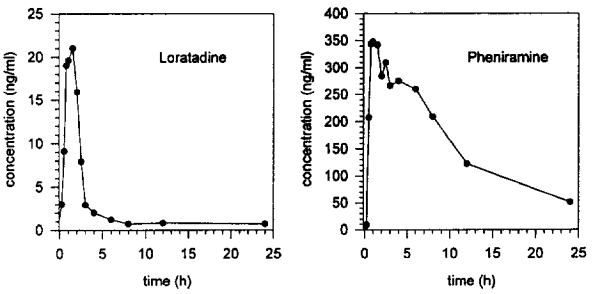

Fig. 4. Serum level time-courses of loratadine and pheniramine after oral intake of 40 mg loratadine or 100 mg pheniramine by healthy volunteers.

4. Application of the assay in clinical trials

Two healthy volunteers have taken an oral dose of 100 mg pheniramine or 40 mg loratadine, respectively. The concentration–time curves of the drugs in serum are shown in Fig. 4. The assay is capable of monitoring the serum levels over a time course of 24 h.

5. Conclusions

The described method is, due to the sophisticated extraction procedure and the applied GC–MS technology, sensitive and selective enough to monitor the low serum levels of the antihistaminic drugs loratadine and pheniramine. It can attend clinical trials as well as the detoxification management in cases of an overdose intake of these drugs.

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