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Fluorimetric method of analysis for D-norpseudoephedrine hydrochloride, glycine and L-glutamic acid by reversed-phase high-performance liquid chromatography

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Abstract

The determination of D-norpseudoephedrine HCl, an appetite suppressant, and glycine and L-glutamic acid, both dietary supplements, in pharmaceutical formulations and dissolution media using reversed-phase high-performance liquid chromatography (HPLC) combined with fluorimetric detection is reported. A reagent solution containing *o*-phthalaldehyde and a reducing agent, mercaptoethanol, appeared to be the most favourable reagent for derivatising the three compounds. The use of this HPLC method allowed for selective and quantitatively accurate analysis and was sufficiently specific, precise and sensitive for analytical characterisation.

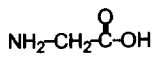
Keywords: Derivatisation, LC; Pharmaceutical analysis; Norpseudoephedrine; Glycine; Glutamic acid

1. Introduction

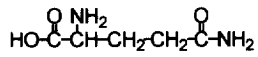
The determination of glycine (aminoacetic acid) and L-glutamic acid (the naturally occurring dextrorotatory form of L-α-aminoglutaric acid with the L-configuration), both dietary supplements, and D-norpseudoephedrine hydrochloride, an appetite suppressant, in pharmaceutical formulations, and dissolution media, presented a problem due to the poor molar absorption of the compounds and retention difficulties. The chemical structures of the compounds are shown in Fig. 1.

During dissolution testing, tablets or capsules, containing the three actives and other ingredients, are placed in 900 ml of a dissolution medium and the rate of dissolution is followed as a function of time. The problem is complicated by the fact that the other actives, for example acetaminophen, which is present in high concentration and has a high molar absorptivity, tends to swamp the peaks of these analytes.

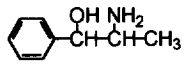
Since the combination of *o*-phthalaldehyde and a



GLYCINE (1)



LEVOGLUTAMIDE (2)



D-NORPSEUDOEPHEDRINE (3)

Fig. 1. The chemical structures of (1) glycine, (2) L-glutamic acid and (3) D-norpseudoephedrine HCl.

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reducing agent appeared to be the most favourable reagent for derivatising amino acids [1], the combination of *o*-phthalaldehyde and mercaptoethanol was tried for the fluorimetric analysis of the three compounds [2,3]. The method was used to quantify these drugs in tablet and liquid formulations. Method validation included range, linearity, precision, accuracy, specificity and recovery [4].

2. Experimental procedures

All chemicals and reagents, including glycine (aminoacetic acid), L-glutamic acid (the naturally occurring dextrorotatory form of L-α-aminoglutaric acid with the L-configuration) and D-norpseudoephedrine hydrochloride were either USP [4] or BP [5] grade and were used without further purification. The drugs were obtained from Sigma (St. Louis, USA). Dosage forms tested were obtained from companies who are selling it in South Africa while some were newly formulated products.

The high-performance liquid chromatographic (HPLC) system consisted of a Shimadzu LC-10A system equipped with a Model LC-10AS pump, RF-551 spectrofluorimetric detector, SIL-10A autosampler, SCL-10A system controller and C-R6A integrator (Shimadzu, Kyoto, Japan). The flow-rate employed was 1 ml min^{-1}. A Phase Sep, Hypersil C_{18} cartridge (Phase Separation, Norwalk, USA; 250×4.6 mm I.D., 5 μm particle size) was used and detector settings were: excitation at 340 nm and emission at 418 nm for D-norpseudoephedrine hydrochloride, excitation at 340 nm and emission at 455 nm for glycine and excitation at 365 nm and emission at 451 nm for L-glutamic acid.

To prepare the mobile phase a buffer solution was prepared containing 0.05 *M* sodium dihydrogen orthophosphate, 0.01 *M* sodium hexane sulphonic acid and 0.0072 *M* triethylamine in deionised water (1000 ml) filtered with a Milli-Q50 system (Millipore, Bedford, USA) and adjusted to pH 3.6 with concentrated phosphoric acid. This solution was prepared by dissolving 6.0 g sodium dihydrogen orthophosphate, 2.06 g sodium hexane sulphonic acid and 1 ml triethylamine in 1 l of water. Optimum mobile phases consisted of a mixture of the buffer and acetonitrile: 40:60 (v/v) for the analysis of D-norpseudoephedrine HCl; 35:65 (v/v) for the analysis of glycine and L-glutamic acid. The isocratic system was operated at ambient temperature, for the analysis of combination preparations the mobile phase was optimised by varying buffer pH and concentration, and organic solvent proportion and type.

To calibrate the method, standard curves were constructed, Table 1, by plotting the peak area of drug against drug concentration. Solutions with known concentrations of drug, alone or combined, were prepared by suitably diluting two standard solutions of each active, containing 120 and 250 μg ml^{-1}, respectively, so as to obtain solutions ranging in concentration of each active between 5 and 25 μg ml^{-1}. These solutions were used to construct standard curves of the actives alone and in combination. Standards and samples were prepared by dissolving the appropriate amount of raw material or powdered tablets/capsules in deionised, filtered water. All solutions were dissolved by means of sonication for 5 min. Samples were filtered through 0.45-μm syringe filters (Millex HA; Millipore, USA) before being placed into sample vials.

Automated on-line sample, pre-column preparation was employed to do the derivatisation reaction. An amount of 200 μl of the sample or standard solution was withdrawn from the vial and dispensed into a mixing bottle. Then 200 μl of pre-prepared derivatisation reagent (Fluoraldehyde; Pierce, Rock-

Table 1
Calibration data for standard drug solutions, showing selectivity and linearity of the method. Results represent the mean values of standards curves constructed from solutions with known concentrations of the drugs alone and in combination

Compound	Concentration range (μg.ml^{-1})	Correlation coefficient	Slope	Standard error of slope	Intercept	Standard error of intercept
D-Norpseudoephedrine HCl	6–24	0.9998	70	3	62	4
Glycine	5–25	0.9991	111	6	21	2
L-Glutamic acid	5–25	0.9998	53	5	12	1

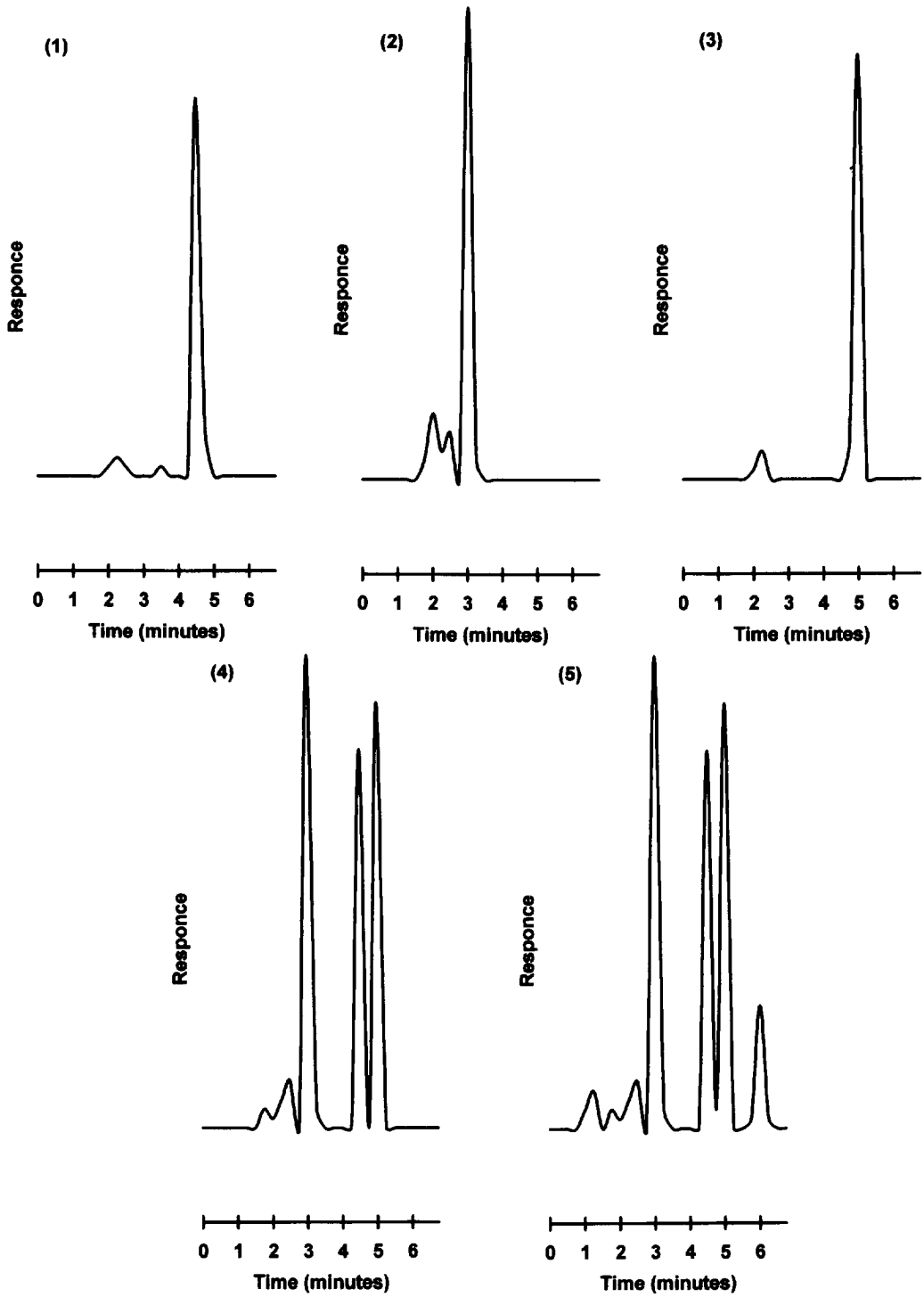

Fig. 2. Chromatograms of (1) glycine, (2) L-glutamic acid and (3) D-norpseudoephedrine HCl, (4) combination of the three drugs and (5) the three drugs in a tablet.

Table 2
Retention times, plate numbers and tailing factors calculated from chromatograms of combinations of the three compounds, Fig. 2(4) [4]

Compound	Retention time (min)	Plate number (N/m)	Tailing factor
D-Norpseudoephedrine HCl	5.0	28727	1.31
Glycine	4.7	7529	1.05
L-Glutamic acid	3.3	17774	1.18

ford, USA: containing 0.8 mg ml^{-1} *o*-phthaldehyde crystals, Brij 35 and mercaptoethanol in a specially formulated borate buffer) was added to the vial, followed by a mixing cycle of five aspirations of 500 μl (including air). An amount of 5 μl was injected onto the column after a 1-min reaction period.

3. Results and discussion

The described mobile phase and chromatographic conditions were sufficient for selective elution of D-norpseudoephedrine HCl, glycine and L-glutamic acid. Separation and analysis required less than 10 min. Chromatograms of the compounds are shown in Fig. 2. As shown in Fig. 2(5), excipients and other actives in pharmaceutical dosage forms, such as tablets containing acetaminophen, did not interfere with the analysis of the three chemicals. However, the method did not distinguish between the D- and L-form of norpseudoephedrine but ephedrine HCl and pseudoephedrine HCl did not interfere with the elution of the D-norpseudoephedrine.

The reproducibility of the chromatographic system was high because the resolution of the compounds of interest (Table 2) did not change over time (Table 3), after repeated injections (Table 4), and throughout the period of testing the retention times of the compounds changed by no more than approximately 1% during a 12 h period. Validation results, including range, linearity, precision, accuracy, specificity and recovery, are presented in Tables 1–5. Pre-column derivatisation of the drugs was optimised by establishing the effect of wavelength (excitation and emission), temperature, reaction time and reagent concentration changes on the analysis of the deriva-

Table 3
Recovery, accuracy and precision of method determined over two days

Compound	Concentration (μg ml^{-1})		Recovery
	Added	Found±S.D.[a]	(%)
D-Norpseudoephedrine HCl	24	23.28±0.14	97.00
Glycine	25	25.68±0.50	102.72
L-Glutamic Acid	25	24.61±0.34	98.44

[a]Based on five samples measured again after 20 h.

Table 4
Recovery of drugs from samples with known concentrations

Compound	Concentration (μg ml^{-1})		Recovery
	Added	Found±S.D.[a]	(%)
D-Norpseudoephedrine HCl	24	23.66±0.77	98.60
Glycine	25	25.43±0.39	101.71
L-Glutamic acid	25	24.54±0.55	98.16

[a]Based on ten replicate analyses of known samples.

Table 5
Precision of the method measured by the analysis of dosage forms

Product	Label claim (mg[a])	Assay (mg[a])	% of label claim (%)	C.V. (%)
D-Norpseudoephedrine HCl tablets	20	20.23	101.15	0.15
D-Norpseudoephedrine HCl syrup	20	19.98	99.90	0.90
Fructomag tablets containing glycine	50	49.60	99.20	0.21
Lentogesic capsules containing L-glutamic acid	25	25.30	101.20	0.79

[a]Units: mg per tablet/capsule, mg per 5 ml for syrup.

tised compounds [1]. Derivatised drugs were then separated using these optimum reaction and analysis conditions as mentioned in the Experimental section.

From calibration plots, the relative correlation coefficients were above $r = 0.999$ for the analysis of the compounds. These linear relationships between peak areas and amounts of products were observed in the range 6–24 μg ml^{-1} for D-norpseudoephedrine HCl and 5–25 μg ml^{-1} for glycine and L-glutamic acid. The lowest recovery after ten replicate injections of known samples (Table 4) was 98.16% and the highest 101.71%. The minimum detectable concentrations, measured by taking the concentration at which the ratio of drug signal to background noise was 3:1, were approximately 0.05 μg ml^{-1} for glycine, 1.0 μg ml^{-1} for L-glutamic acid and 2.5 μg ml^{-1} for D-norpseudoephedrine HCl. Results obtained after replicate injection of the same solutions gave a precision of better than 1% (Table 3 and Table 4). Inter-day repeatability (Table 3) was also excellent.

These results illustrated that the described reversed-phase HPLC method was both accurate, precise and could be repeated without excipients or other active ingredients (Table 5) interfering with the analyte peaks. The use of the method combined with automated derivatisation and fluorimetric detection therefore allowed for the selective and quantitatively accurate analysis of D-norpseudoephedrine HCl, glycine and L-glutamic acid in pharmaceutical dosage forms, i.e. tablets or syrups. The chromatographic method was also sufficiently specific, precise and sensitive for the purpose of analytical characterisation, while on-line automated derivatisation made the method less time consuming.

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