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Short communication

Determination of citalopram and escitalopram together with their active main metabolites desmethyl(es-)citalopram in human serum by column-switching high performance liquid chromatography (HPLC) and spectrophotometric detection

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Abstract

We established a method for automated quantitative analysis of (es-)citalopram and desmethyl(es-)citalopram in serum using column-switching high performance liquid chromatography (HPLC). For sample clean-up serum was injected onto a LiChrospher CN 20 μm precolumn using 8% acetonitrile in deionized water. Drugs were eluted by back-flush flow onto the analytical column (LiChrospher CN 5 μm) at a flow rate of 1.5 ml/min with phosphate buffer 8 mmol/l pH 6.4/acetonitrile (50/50, v/v). Haloperidol was used as internal standard. Analytes were detected by ultraviolet spectrophotometry at 210 nm. Detection limit of (es-)citalopram was 6 ng/ml. The method was found to be suitable for therapeutic drug monitoring of patients treated with citalopram or escitalopram.

Keywords: Citalopram; Escitalopram; Quantitative analysis; HPLC column-switching; TDM

1. Introduction

Citalopram is chemically a bicyclic phthalate and a selective serotonin reuptake inhibitor (SSRI). It is a racemic drug used for the treatment of depression with the *S*-enantiomer being the pharmacologically active compound. Compared to other SSRIs (es-)citalopram has proven to be the most selective of all, concerning the neuronal reuptake inhibition of serotonin There is no influence on the neuronal reuptake of noradrenaline and dopamine. Anticholinergic and antihistaminergic effects, responsible for typical side effects of tricyclic antidepressants, are lacking, dopaminergic, adrenergic and GABA effects are minimal. (Es-)citalopram is metabolized by *N*-demethylation to desmethyl(es-)citalopram, which can also inhibit 5-HT reuptake, but its potency is much weaker than the potency of the parent substance [1]. A high bioavailability (>90%) of (es-)citalopram is characteristic for the substance.

(Es-)citalopram is licensed for the treatment of depressive and panic disorders with and without agoraphobia. Recommended daily doses range from 20 to 40 mg of citalopram and from 10 to 20 mg of escitalopram, corresponding drug concentrations were detected between 30 and 130 ng/ml [2] and 15–80 ng/ml, respectively [3]. Blood concentrations show high inter- and intra-individual variations. For this reason, routine measurement of (es-)citalopram has been recommended as useful (level 3 of the AGNP–TDM consensus guidelines) [4,5].

Analytical methods based on high performance liquid chromatography (HPLC) are most often reported for the quantitative determination of citalopram [6–12]. All of the so far published methods require a sample preparation before transferring the sample onto the analytical system. Such an approach is time consuming and reduces sensitivity because of the loss of material. Today, progress in analytical instrumentation allows on-line extraction of the drug by HPLC with column-switching oper-

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ated in back-flush mode. This paper describes such a method that yields a fully automated procedure.

There is no report so far on the quantitative determination of escitalopram. However, since escitalopram is the *S*-enantiomer of citalopram, chromatographic characteristics in an achiral environment are the same, so that the method may be also used for its quantitative determination as long as no qualitative differentiation between racemate and enantiomer is necessary.

2. Conditions

2.1.1. Chemicals

Citalopram hydrobromide (LU 10-171-B), escitalopram–oxalate (LU 26-054-O) and desmethylcitalopram hydrochloride (LU 11-109-C) were kindly supplied by Lundbeck (Copenhagen/Denmark). Haloperidol as an internal standard was obtained from Janssen–Cilag GmbH (Beerse/Belgium).

Di-potassium-hydrogenphosphatetrihydrat LiChropur® and ortho-phosphoric acid Suprapur® 85% were obtained from Merck KGaA (Darmstadt, Germany); Acetonitrile Ultra Gradient HPLC grade from J.T.Baker (Mallinckrodt Baker B.V., Deventer/Holland).

Human drug-free serum was prepared from whole blood drawn from healthy volunteers (Bezirksklinikum Regensburg, Germany).

2.1.2. Sample preparation

Stock solutions of each analyte were prepared by solubilising 10 mg citalopram hydrobromide and 10 mg desmethylcitalopram hydrochloride, respectively, in 10 ml of methanol. The stock solutions were diluted by deionized water to 1 μg/ml. Further dilution steps (400–200–100–50–25–12.5 ng/ml) were performed by:

- precolumn eluent (acetonitrile 8% in deionized water) for determination of the drugs in validation experiments
- by drug-free human serum obtained from healthy volunteers.

The internal standard was prepared by dissolving 5 mg haloperidol in 10 ml of methanol. 10 μl of this stock solution were mixed with 990 μl precolumn eluent resulting in a concentration of 5 μg/ml. 5 μl of this solution (25 ng) were automatically added to each specimen by the autosampler.

To set up precolumn eluent (8% acetonitrile in water), 160 ml acetonitrile were supplemented to a volume of 2000 ml with deionized water. The mixture was degassed in an ultrasonic bath for 5 min. Precolumn eluent was also used to rinse the injection needle of the autosampler between injections in order to avoid carry-over effects by adhering proteins or drug remnants.

For preparation of analytical eluent, 8 mmol/l phosphate buffer (3.65 g di-potassium-hydrogenphosphatetrihydrate in 2000 ml deionized water) pH 6.4 was mixed with acetonitrile 50/50 (%v/v), the pH-value was corrected with ortho-phosphoric acid to pH 6.4 and the obtained solution was degassed in an ultrasonic bath.

Serum samples of patients treated with (es-)citalopram were sent to the laboratory and centrifuged directly after delivery for 10 min at 4000 U/min. Samples were then either rapidly analysed or stored at −20 °C.

2.1.3. Instrumentation

Sample preparation and the analytical procedure were carried out on a single component HPLC system (Dionex GmbH, Idstein, Germany) consisting of a GINA 50 autosampler, an isocratic low pressure pump P680 for the precolumn eluent, and another low pressure gradient pump LPG 680 to deliver the analytical eluent to the analytical column. The process of switching from the precolumn to the analytical column was executed by an electric six-port valve (Electronic Valve Actuator). Drugs were detected by a variable photodiode array UV-detector PDA-100 set to 210 nm, differing from the absorption maximum of (es-) citalopram ($lambda_{max.}$ 240 nm). The choice of this wavelength resulted in higher signal areas, which was essential to obtain a lower limit of detection of the analytes in this method. The chromatogram was evaluated and integrated by the Chromeleon software Version 6.3 SP1 Build 587.

The precolumn was a LiChrospher CN 20 μm, 10 × 2.1 mm; a CN-endcapped LiChrospher 250 × 4.6 mm, 5 μm, was used as analytical column (both obtained from MZ-Analysentechnik, Mainz, Germany).

2.1.4. On-line extraction and chromatographic procedure

After injection of 245 μl serum + 5 μl internal standard into the HPLC system (start of data acquisition) each sample was transferred onto the precolumn by precolumn eluent at a flow rate of 1.5 ml/min with the electric six-port valve set to the extraction position for 5 min. Because of their high polarity, matrix constituents of the serum samples were not retained on the precolumn but eluted to waste. Less polar molecules such as the drugs under investigation were retained and thereby concentrated on top of the precolumn. The retained drugs were flushed off the precolumn by the analytical eluent in back-flush mode at a flow rate 1.5 ml/min for 5–25 min of the analytical run after switching the electric motor valve to the separation position. The analytical eluent was recycled for one week. At 25 min the motor valve was again switched to the extraction position, data acquisition was stopped, and the cleaning programme was started by injection of 250 μl precolumn eluent. At 30 min the HPLC system was ready to receive the next specimen.

2.2. Quantification and validation of the method

Quantification was based on calibration of peak areas. Peak areas of the analytes were normalized to the peak area of the internal standard. The method was validated by documenting its linearity, limit of detection and precision as well as the recovery of citalopram and its metabolite from human serum. Each data point was documented in triplicates.

Precision was computed from the coefficient of variation of serum samples in a concentration range between 12.5 and

200 ng/ml. Intraday precision was defined as precision within all determinations analysed in one day, interday precision as precision between individual runs on different days.

Linearity of serum spiked with citalopram and desmethylcitalopram was documented in dilution experiments over five concentrations (12.5–25–50–100–200 ng/ml serum).

Recovery was determined in three different drug concentrations (12.5–50–200 ng/ml) as compared to aqueous solutions of the drugs analysed, set to 100%.

The *limit of detection* was determined as the concentration that yielded a peak area three times of baseline instability.

Values are specified as mean ± standard deviation (SD) unless otherwise indicated.

3. Results

3.1. Retention times

The retention time of desmethylcitalopram with eluent in recycling mode was 20.0 ± 0.61 min, that of citalopram 21.6 ± 0.80 min. Both substances could be clearly separated. The internal standard haloperidol was eluted at 24.3 ± 1.01 min and was thus well separated from the peaks of the analytes (Fig. 1). The run was terminated at 25 min.

Variation of retention times can be simply decreased by flushing the analytical eluent to waste: experiments have shown faster analytical runs and retention times for citalopram, desmethylcitalopram and haloperidol with retention times decreased to 12.04 ± 0.22 min, 11.46 ± 0.11 min and 13.78 ± 0.08 min, respectively.

3.2. Precision

Intraday coefficient of variation within the analysed concentration range was always below 7% for citalopram and below 1% for desmethylcitalopram

Interday variation was <9.05% for citalopram and <14.88% for desmethylcitalopram (Table 1).

3.3. Linearity

The calibration curves of both citalopram and its active metabolite in serum were linear over the whole concentration range investigated. This result was confirmed by linear regression analysis with correlation coefficients (r) above 0.99 (Table 1).

3.4. Recovery

Recovery was 84 ± 0.6% for 200 ng/ml and 73 ± 0.7% for 50 ng/ml citalopram, 74 ± 0.1% for 200 ng/ml and 64 ± 1.5% for 50 ng/ml desmethylcitalopram. Coefficients of variation for citalopram were lower at 200 ng/ml than at 50 ng/ml and increased 10-fold at concentrations of 12.5 ng/ml (74 ± 6.4%). For desmethylcitalopram, recovery at 12.5 ng/ml was 87 ± 1.9%.

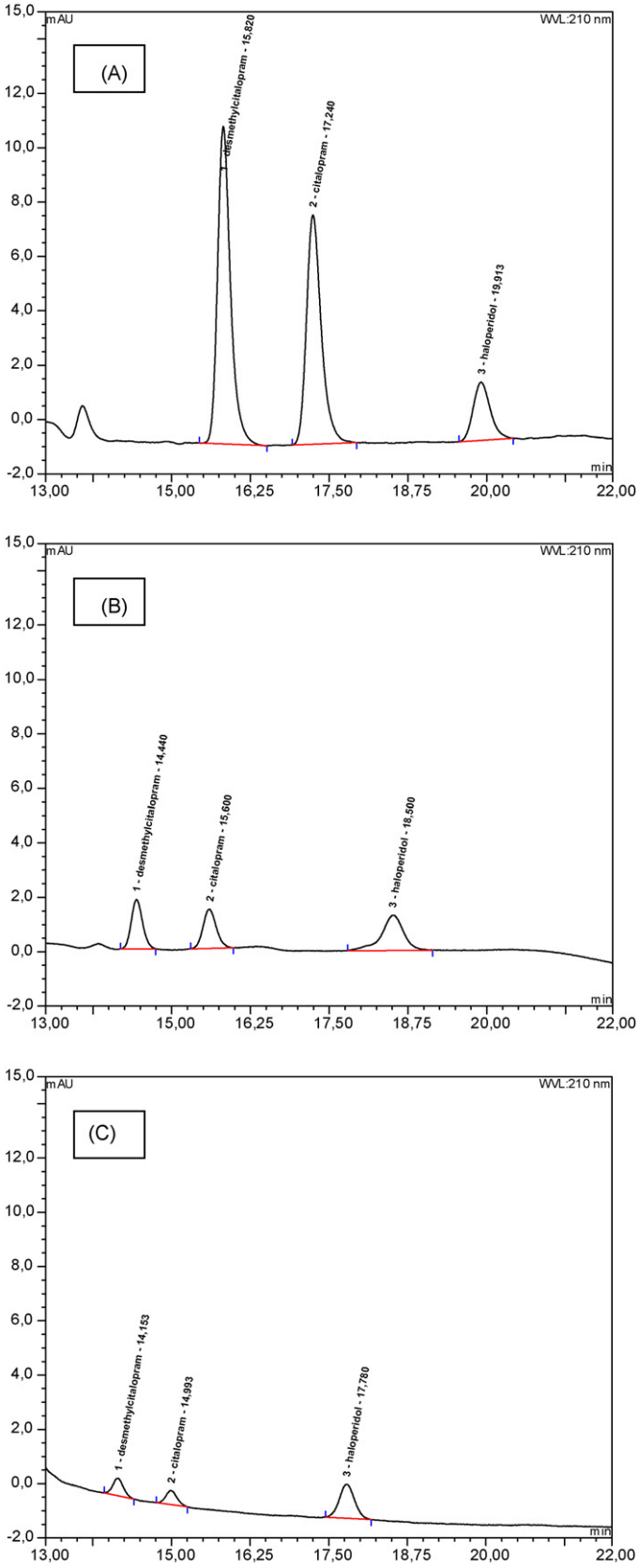

Fig. 1. Representative chromatograms showing the analytes dissolved in eluent (A) and serum (B) spiked with 50 ng/ml citalopram and desmethylcitalopram and 25 ng/ml haloperidol each. Chromatogram obtained from a patient after a daily citalopram dose of 20 mg with a corresponding concentration of 14 ng/ml (C).

Table 1
Calculations on precision (within run and between run variation of concentration) of human serum spiked with citalopram and desmethylcitalopram depicted by coefficients of variation (CV) in [%] and linearity of the assay depicted by the equation of the linear regression curves and correlation coefficients (r)

	Concentration added (ng/ml)	Citalopram	Desmethylcitalopram
Within run precision (CV) in [%]	200	0.1878	0.0443
	50	0.6069	0.2488
	12.5	6.2807	0.0012
Between run precision (CV) in [%]	200	3.9111	6.5040
	50	6.1589	3.5707
	12.5	9.0520	14.8816
Equation of linearity ($y = a \times x + b$) correlation coefficient (r)		$(y = 0.0081x - 0.0352) \geq 0.9969$	$(y = 0.0084x - 0.0273) \geq 0.9947$

3.5. Limit of detection

The limit of detection was 6.25 ng/ml for both, citalopram and its metabolite desmethylcitalopram. These findings were in accordance with a signal-to-noise ratio of 3:1. A calculation ($n = 7$) following the determination of precision and recovery of citalopram revealed, that the coefficient of variation was below 20% at this concentration.

3.6. Application to patient samples

The method was suitable for determination of citalopram and desmethylcitalopram concentrations in serum of patients treated with citalopram or escitalopram (Fig. 1). When using for therapeutic drug monitoring in clinical routine, the precolumn had to be replaced after 50 serum injections due to an increase of backpressure. The analytical column showed retention shifts with increasing backpressures up to 200 bar after 300–500 injections of 245 μl of serum.

4. Conclusion and discussion

A simple HPLC method has been developed for a sensitive determination of citalopram and its metabolite desmethylcitalopram over their entire therapeutic range, using the advantages of an on-line sample preparation by column-switching procedure. Serum samples containing citalopram and desmethylcitalopram could be separated by injection of 245 μl within 20 min including precleaning of the sample. Though the absorption maximum (lambda max.) of (es-)citalopram is 240 nm, we used 210 nm due to twofold higher signal areas, which was essential to obtain a sufficiently low limit of detection of the analytes in this method. Intraday variations within the analysed concentration levels were always below 2% for both drugs indicating high precision of the method. An imprecision of up to 15% for both drugs among interday measurement experiments is considered acceptable for therapeutic drug monitoring of psychotropic drugs [13]. Our method is both, cost and time effective. The analytical eluent was recycled within the analytical run and renewed once a week and by saving reagent materials formerly used for sample preparation. Monitoring of a single specimen suffices in case of suspected overdosage intoxications reporting a result within half an hour. The method may be extended to other drugs having a similar structure without modification. Reducing the injection volume in order to increase the column lifetime by preventing from clocking pores with endogenous serum components led to higher detection limits of citalopram and desmethylcitalopram because of decreased signal areas. Therefore columns based on monolithic material could be an alternative for detecting low concentrations of analytes by means of excluding proteins in the void volume and doing without frits at the ends of the column body, where adsorption can occur.

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References

[1] P. Popik, J. Clin. Psychopharmacol. 19 (5 Suppl. 1) (1999) 4.
[2] L. Bjerkenstedt, L. Flyckt, K.F. Overo, O. Lingjaerde, Eur. J. Clin. Pharmacol. 28 (5) (1985) 553.
[3] Summary of product characteristics (SPC): cipralex®, March 2006.
[4] M. Reis, G. Olsson, B. Carlsson, J. Lundmark, M.L. Dahl, J. Walinder, J. Ahlner, F. Bengtsson, J. Clin. Psychopharmacol. 22 (4) (2002) 406.
[5] P. Baumann, C. Hiemke, S. Ulrich, G. Eckermann, et al., Pharmacopsychiatry 37 (2004) 243.
[6] K.K. Akerman, J. Jolkkonen, H. Huttunen, I. Penttila, Ther. Drug Monit. 20 (1) (1998) 25.
[7] J. Macek, P. Ptacek, J. Klima, J. Chromatogr. B Biomed. Sci. Appl. 755 (1–2) (2001) 279.
[8] G. Tournel, N. Houdret, V. Hedouin, M. Deveau, D. Gosset, M. Lhermitte, J. Chromatogr. B Biomed. Sci. Appl. 761 (2) (2001) 147.
[9] R. Waschgler, M.R. Hubmann, A. Conca, W. Moll, P. Konig, Int. J. Clin. Pharmacol. Ther. 40 (12) (2002) 554.
[10] L. Kristoffersen, A. Bugge, E. Lundanes, L. Slordal, J. Chromatogr. B Biomed. Sci. Appl. 734 (2) (1999) 229.
[11] K. Titier, N. Castaing, E. Scotto-Gomez, F. Pehourcq, N. Moore, M. Molimard, Ther. Drug Monit. 25 (5) (2003) 581.
[12] C. Duverneuil, G.L. de la Grandmaison, P. de Mazancourt, J.C. Alvarez, Ther. Drug Monit. 25 (5) (2003) 565.
[13] ICH expert working group: Q2(R1): validation of analytical procedures: text and methodology, published in the Federal Register, vol. 60, 1995, pages 11260.