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Determination of cholesterol oxides in processed food using high-performance liquid chromatography–mass spectrometry with atmospheric pressure chemical ionisation

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Abstract

The present work describes the development and application of an on-line atmospheric pressure ionisation (APCI) LC–MS interface for the simultaneous determination of seven toxicologically relevant cholesterol oxides (7α-hydroxycholesterol, 7β-hydroxycholesterol, 25-hydroxycholesterol, 7-ketocholesterol, 5,6α-, 5,6β-epoxycholesterol and cholestan-3β,5α,6β-triol). The HPLC method has been optimised to reach better separation of all tested compounds. The influences of APCI parameters (nebulising temperature, cone voltage, source temperature) on signal intensity and fragmentation pattern were investigated for all tested cholesterol oxides compounds. This is the first report on optimisation and determination of two compounds 7α-hydroxycholesterol and 5,6β-epoxycholesterol in processed food using LC–MS. After extraction with hexane, clean-up was carried out using solid-phase extraction on a silica column. For the chromatographic separation of cholesterol oxides an Aquasil C_{18} column was used with acetonitrile–methanol (60:40) as mobile phase. For the first time we report the use of such a C_{18} column with a relatively hydrophilic nature for the separation of cholesterol oxides. APCI-MS detection was then applied in selected ion monitoring and positive ion modes by using the molecular ions and the main fragments. The developed method shows good linearity, high repeatability and good recovery for all tested cholesterol oxides. The method was applied for determination of seven selected cholesterol oxidation products in different foodstuffs such as butter, butteroil, lard and egg powder.

Keywords: Butter; Oils; Food analysis; Cholesterol oxides

1. Introduction

Recently the oxidation of cholesterol in processed food has received considerable attention due to the well known toxicological relevance of the autooxidation products. Cholesterol is an unsaturated alcohol, susceptible to oxidation in the presence of light, oxygen and high storage temperature [1,2]. This autooxidation involves a free-radical reaction, which leads to the formation of more than 60 different oxidation products [3,4]. The primary autooxidation process, most frequently results in the formation of hydroperoxides in the B-ring and the side chain and, to a lesser extent, in the dehydrogenation of the 3β-alcohol group. These hydroperoxides are unstable and could decompose to secondary oxidation products, which have different chemical functional

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groups such as hydroxy, keto and epoxy [2]. Components such as 7α-hydroxycholesterol (7α-OH), 7β-hydroxycholesterol (7β-OH), 25-hydroxycholesterol (25-OH), cholestan-3β,5α,6β-triol (triol), 7-ketocholesterol (7-keto), 5,6α-epoxycholesterol (5,6α-EP) and 5,6β-epoxycholesterol (5,6β-EP) are some major cholesterol oxidation products (COPs). These substances also called ''oxysterols'' can be found in many cholesterol-containing foodstuffs like milk and dairy products [5–9], meat and meat products [10–15] as well as egg products [16–21]. Especially, technological processes and treatment such as heating, irradiation and storage can increase cholesterol oxidation [2].

Cholesterol oxidation products turned out to have a variety of potentially atherogenic, cytotoxic, mutagenic and possibly carcinogenic effects in both in vivo and in vitro studies [22–27]. Several investigations demonstrated that 25-hydroxycholesterol and triol are toxic agents causing atherosclerosis [2,22–27]. Also 5,6α-epoxycholesterol and 5,6β-epoxycholesterol have been reported to be carcenogenic [2,24–29].

The possible health implications of dietary cholesterol oxides have stimulated the necessity for identification and determination of these components in food. Due to the large amounts of triglycerides and phospholipids, the isolation, separation and detection procedures of cholesterol oxides in foods at low concentrations (μg/kg) are difficult analytical problems. Additionally the COPs have very similar chemical structures, so that the separation of all toxicologically interesting COPs is not easy to achieve.

Several analytical methods such as thin-layer chromatography (TLC) [30–33] and gas chromatography (GC) [17,18,34–39] have been developed for determination of cholesterol oxides in foodstuffs. High-performance liquid chromatography (HPLC) methods using UV, high-resolution nuclear magnetic resonance (NMR) and refraction index, have been applied for the separation and quantification of oxysterols by several researchers [40–49]. The use of UV detection is limited due to the poor and unspecified absorption of some oxysterols like 5,6α-epoxycholesterol, 5,6β-epoxycholesterol as well as cholestan-3β,5α,6β-triol [40,41]. Therefore the analysis of COPs with UV detection should be applied in the low UV range (206 nm) which is a difficult task if considering the determination of cholesterol oxides in biological matrices in low-level concentration.

Most recent analytical methods for the identification and quantification of cholesterol oxides are based on capillary GC combined with mass spectrometry (MS) [13,50–52] which offers better sensitivity. The use of GC requires the circumstantial procedure of saponification of lipidic extract and the subsequent extraction of the unsaponified fraction containing cholesterol oxides. All these time consuming steps and additionally the derivatisation may lead to a reduction of recovery and formation of artefacts [37].

The combination of HPLC with MS holds great promise for the trace determination of substances which are either too polar or too labile to be analysed by GC–MS without derivatisation. Especially, an atmospheric pressure ionisation (APCI) interface seems to be a powerful tool for the analysis of COPs in biological matrices. The LC–MS analysis of COPs was reported by Careri et al. [53] using a particle beam interface. In a recent work five oxidation products of cholesterol were analysed by LC–APCI-MS in a lyophilised beef sample [54].

The present work describes a method for extraction, purification and analysis of seven toxicological relevant cholesterol oxides in different foodstuffs by HPLC–MS with an APCI interface. The HPLC method was improved to obtain better separation for all tested compounds. The influences of APCI parameters (nebulising temperature, cone voltage) on signal response and fragmentation pattern were investigated. The linearity, detection limits, inter-assay precision, intra-assay precision and recoveries were determined for all tested compounds.

All seven toxicologically relevant COPs could be separated and determined simultaneously using HPLC in combination with MS. Additionally to previous works [52,54] we describe, for the first time, the optimisation and quantitative determination of 7α-hydroxycholesterol and 5,6β-epoxycholesterol using an APCI interface. The developed method was applied to the analysis of COP contamination of different processed foods. This work also shows, in comparison to Manini et al.'s investigations, how reproducible and the comparable spectra and the optima in two different APCI interfaces are.

2. Materials and methods

2.1. Chemicals and reagents

Acetonitrile (LiChrosolv) and methanol (LiChrosolv) used in HPLC as well as chloroform, *n*-hexane, diethyl ether, ethyl acetate and acetone were from Merck (Darmstadt, Germany). Buffer salts of analytical grade were also obtained from Merck. All solvents used in HPLC–MS were filtered through a 0.2-μm filter to remove particles and were degassed using a Waters in-line degasser (Milford, MA, USA). The COP standards were purchased from Sigma (St. Louis, MO, USA).

2.2. HPLC–MS equipment

The LC system used in the HPLC–MS experiments consisted of a Waters 626-LC pump and a Waters 717plus autosampler. Chromatographic separation and detection were performed on a Quattro II instrument using an APCI interface equipped with a Pepperpot counter electrode (Micromass, Manchester, UK).

As analytical column an Aquasil C_{18}, 250 mm× 4.6 mm, 5 μm (Keystone Scientific, Bellefonte, PA, USA) was used and column temperature was kept at 25°C. The isocratic mobile phase consisted of acetonitrile–methanol (60:40, v/v) with a flow-rate of 1 ml/min without splitting.

The mass spectrometric detection was performed in positive ion mode (APCI). The optimisation steps were carried out in flow injection mode using a scan range of *m/z* 250–500 (scan time of 1 s and interscan delay of 0.1 s) by three repetitive injections for each point. Nitrogen of pure quality as nebulising and carrier gas was used (≥99.996%, Linde, Munich, Germany). Carrier gas flow was set at 250 l/h and sheath gas flow was held at 50 l/h.

The source temperature was kept at 175°C, the APCI vaporising temperature was held at 500°C and the cone voltage was set at 20 V. Quantitative determination of all compounds was applied in the single ion monitoring (SIM) mode using *m/z* 401.5 for 7-keto and *m/z* 385.5+367.5 for all other COPs. The ions in SIM mode have been detected in a dwell time of 0.3 s and span of 0.2 u.

2.3. Extraction

Sample clean-up steps were performed using solid-phase extraction (SPE) cartridges after extraction with appropriate organic solvents.

Butter (5.0 g) and butteroil (5.0 g) as well as lard samples (5.0 g) were melted in a heater at 40°C. After filtering, exactly 2 g of melted fat was transferred to a beaker and dissolved in 3 ml hexane. This solution was loaded then onto the SPE column.

Egg powder (1.0 g) was extracted with 30 ml chloroform using an Ultra-Turrax homogeniser (Janke & Kunkel IKA-Labortechnik, Staufen, Germany) for 1 min. After filtration over sodium sulfate, the extract was dried in a rotary evaporator (Büchi, Flawil, Switzerland) at 45°C and the residue was dissolved in 5 ml hexane and loaded onto the SPE column.

2.4. Sample preparation on SPE

The COPs were separated from the matrix using 3 ml (500 mg) SPE silica cartridges (International Sorbent Technology, Mid-Glamorgan, UK). The SPE columns were pre-conditioned with 3 ml hexane prior to loading the hexanic extract solution onto the silica columns. Afterwards the SPE columns were washed three times with the following reagents: in the first step 10 ml hexane–diethyl ether (95:5, v/v), in the second step 25 ml hexane–diethyl ether (90:10, v/v) and in the third step 15 ml hexane–diethyl ether (80:20, v/v). The COPs were then eluted with 5 ml acetone. The acetone was evaporated under nitrogen, the residue was dissolved in 1 ml of mobile phase and 50 μl was injected into the LC–MS system.

3. Results and discussion

3.1. Mass spectra and APCI fragmentation

The mass spectra of the tested COPs are shown in Fig. 1 and the relative abundance of each fragment is given in Table 1. They were recorded in full scan mode (*m/z* 250–500). As can be seen in Fig. 1, similar fragmentation patterns were observed for six of tested substances, this fact revealed the necessity

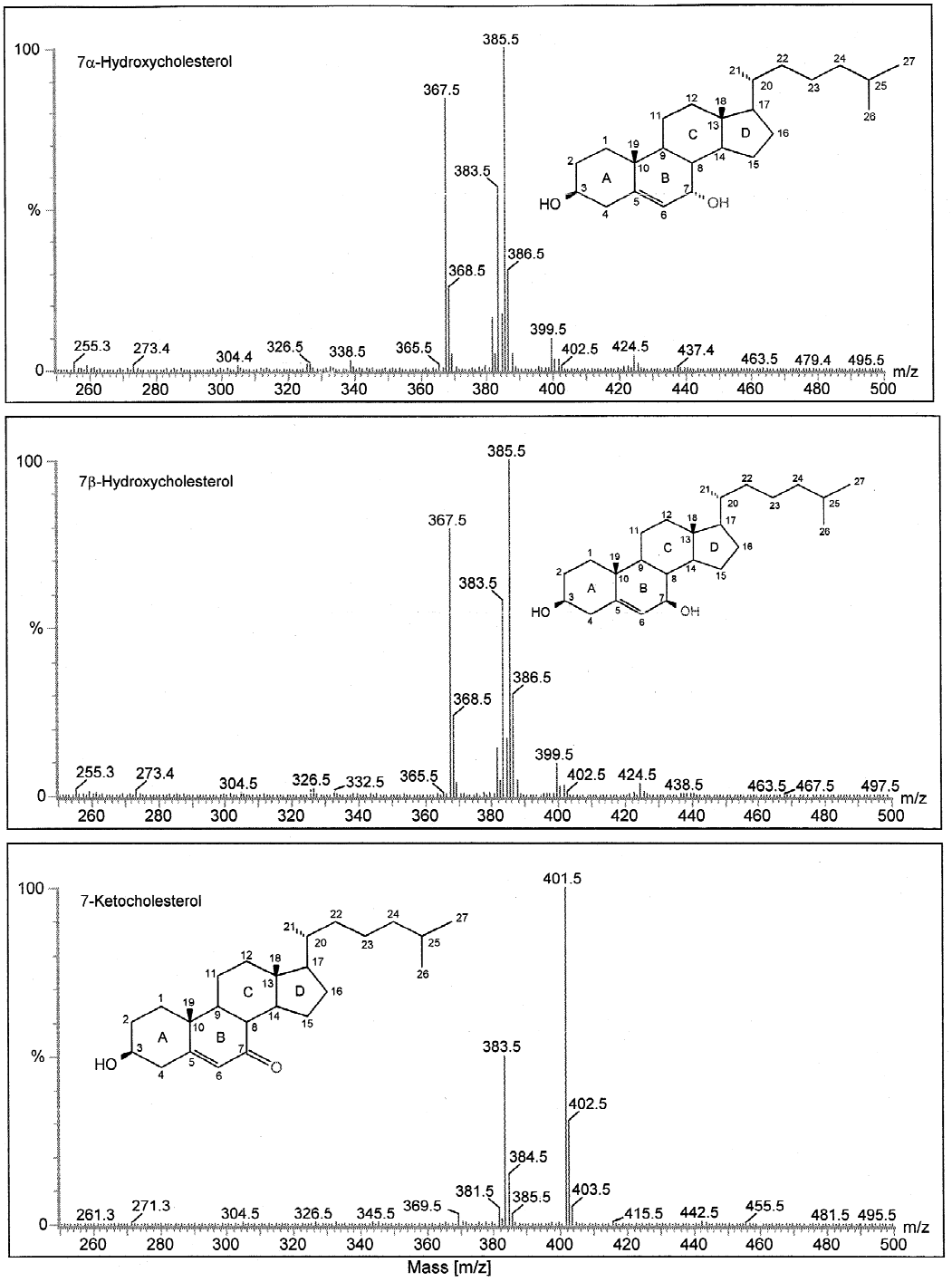

Fig. 1. Mass spectra and the appropriate chemical structure of each tested cholesterol oxide in the positive ionisation mode.

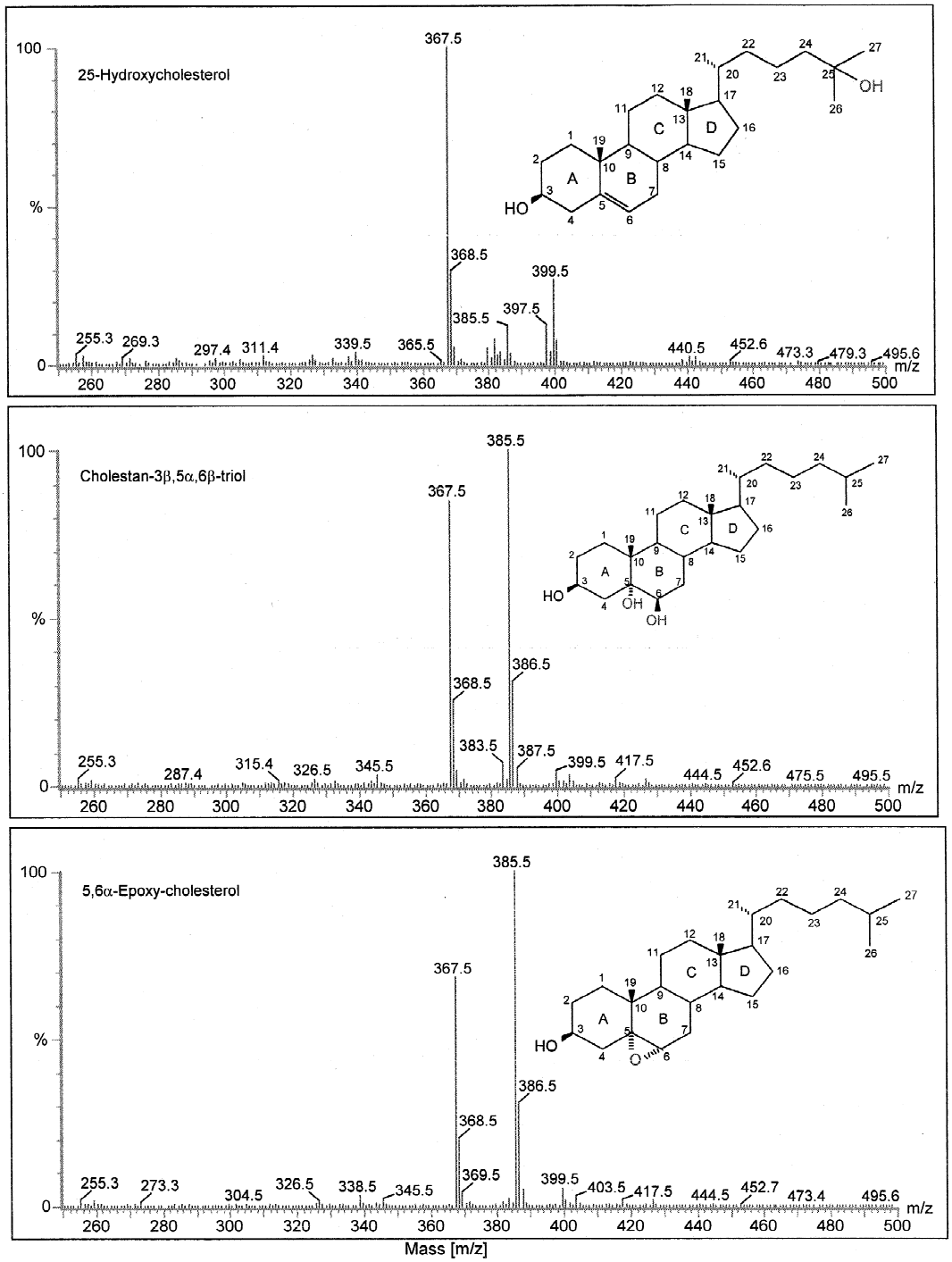

Fig. 1. (*continued*).

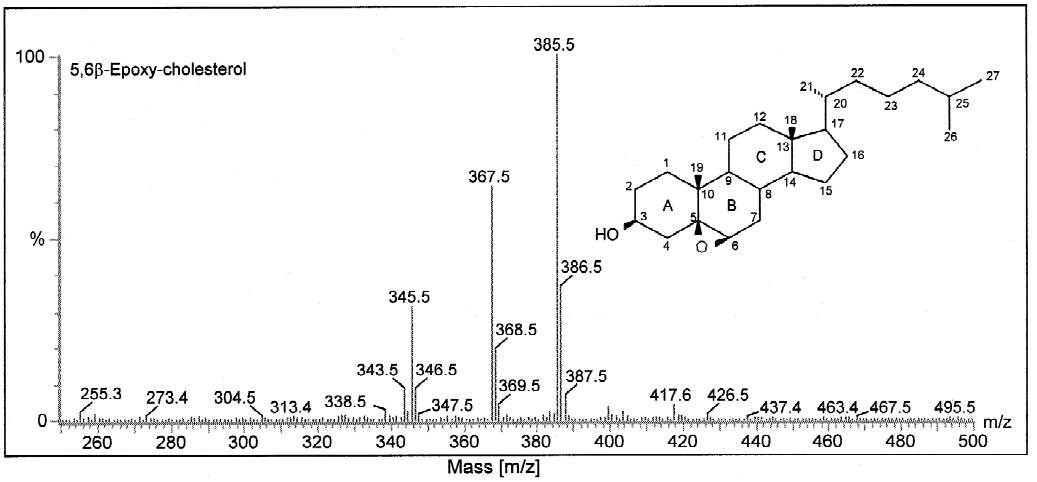

Fig. 1. (*continued*).

of an appropriate separation of COPs in chromatography. Only in the case of 7-ketocholesterol the base peak of protonated molecule could be detected *m/z* 401.5 $[M+H]^+$, having the highest abundance, which corresponds to the results of Manini et al. [54]. This advantage could be taken for the mass separation of 7-keto and 25-OH, since the *m/z* 401.5 mass was not detectable in any other mass spectra of COPs. For all the other analysed cholesterol oxides no molecule ions have been observed. In general the fragment ions *m/z* 385.5 $[M+H-H_2O]^+$, *m/z* 367.5 $[M+H-2H_2O]^+$ were observed, which indicate the loss of one or two molecules of water. Also adduct ions of *m/z* 399.5 $[M+H-H_2O+CH_3OH]^+$ could be detected for all COPs except 7-keto. In the case of 25-OH the intensity of the *m/z* 385.5 fragment was much lower than in the other cases. For triol with M_r 420.6, the loss of one and two water molecules led to the following fragments *m/z* 385.5 $[M+H-2H_2O]^+$, *m/z* 367.5 $[M+H-3H_2O]^+$. But no *m/z* 403.5 fragment ion was observed, which is not comparable to Manini et al.'s results [54]. Also in contrast, no molecular ion has been observed in case of α-EP. In comparison to mass spectra described by Manini et al. some differences in ionisation and fragmentation behaviour of COPs could be recorded, which can be explained by the different interface design.

3.2. Optimisation of APCI parameters

The different operational parameters of the APCI interface and MS were optimised for the seven compounds under study and those presenting the maximum sensitivity were chosen for further analysis. The most relevant parameters were the APCI probe temperature, cone voltage and ion source temperature. Since in this work the interface design of Micromass APCI interface is different to those

Table 1
Molecular mass molecular ions and major fragments of tested COPs

Compound	Abbreviation	M_r	Main fragments (rel. abundance)
7α-Hydroxycholesterol	7α-OH	402.6	385.5 (100%), 367.5 (79%), 383.5 (54%), 386 (29%)
7β-Hydroxycholesterol	7β-OH	402.6	385.5 (100%), 367.5 (80%), 383.5 (56%), 386 (30%)
25-Hydroxycholesterol	25-OH	402.6	367.5 (100%), 368.5 (30%), 399.5 (26%)
7-Ketocholesterol	7-Keto	400.6	401.5 (100%), 383.5 (50%), 402.5 (31%)
Cholestan-3β,5α,6β-triol	Triol	420.6	385.5 (100%), 367.5 (85%), 386.5 (3 1%)
5,6α-Epoxycholesterol	α-EP	402.6	385.5 (100%), 367.5 (68%), 386.5 (3 1%)
5,6β-Epoxycholesterol	β-EP	402.6	385.5 (100%), 367.5 (63%), 386.5 (37%), 345.5 (31%)

used by Manini et al. [54], we decided to optimise MS parameter for all seven toxicologically relevant COPs. Additionally we studied the effects of interface parameter on two further oxysterols 7α-hydroxycholesterol and 5,6β-epoxycholesterol, because no data about the optimum conditions of β-EP and β-OH in the APCI interface have been published yet.

3.2.1. Effect of vaporiser temperature

In a first step the effect of vaporiser temperature on signal intensity of tested cholesterol oxides was studied by varying the probe temperature between 200 and 550°C in full scan mode (*m*/*z* 120–600 u). 7-Keto, 7α-OH and 7β-OH could be ionised and detected in low temperature range (Fig. 2) of 200°C. An optimum for 7α-OH could be reached at 450°C, whereas for 7β-OH and 7-keto best signal was recorded at 400°C. The maximum response for 25-OH and triol was attained at 550°C and in the case of α-EP and β-EP 500°C was observed to be the best. The results are similar to those obtained by Manini et al. but not the same. Fig. 2 shows the effect of vaporiser temperature on signal intensity of 7α-hydroxycholesterol and 5,6β-epoxycholesterol. No changes in fragmentation pattern of tested substances could be observed over the applied temperature range 200–550°C. This fact indicates that the fragmentation is not the result of a thermal degradation.

3.2.2. Effect of cone voltage

The second step was to optimise the cone voltage and study the effect on fragmentation pattern of COPs. Additionally to Manini et al.'s work, we studied the effect of cone voltage on signal intensity of 7α-hydroxycholesterol and 5,6β-epoxycholesterol, which is shown in Fig. 3. A better sensitivity for 7β-OH and 7α-OH was achieved at low voltages, whereas for 7-keto, a better response was obtained at higher voltages of about 30 V, without any changes of fragmentation pattern between 10 and 30 V. Above 30 V a decrease of signal intensity was observed for 7-keto. In the case of α-EP and β-EP the highest abundance could be found in a range between 15 and 20 V. Triol and 25-OH had the best sensitivity at 20 V. To reach the best sensitivity for all seven tested COPs in quantitative determination,

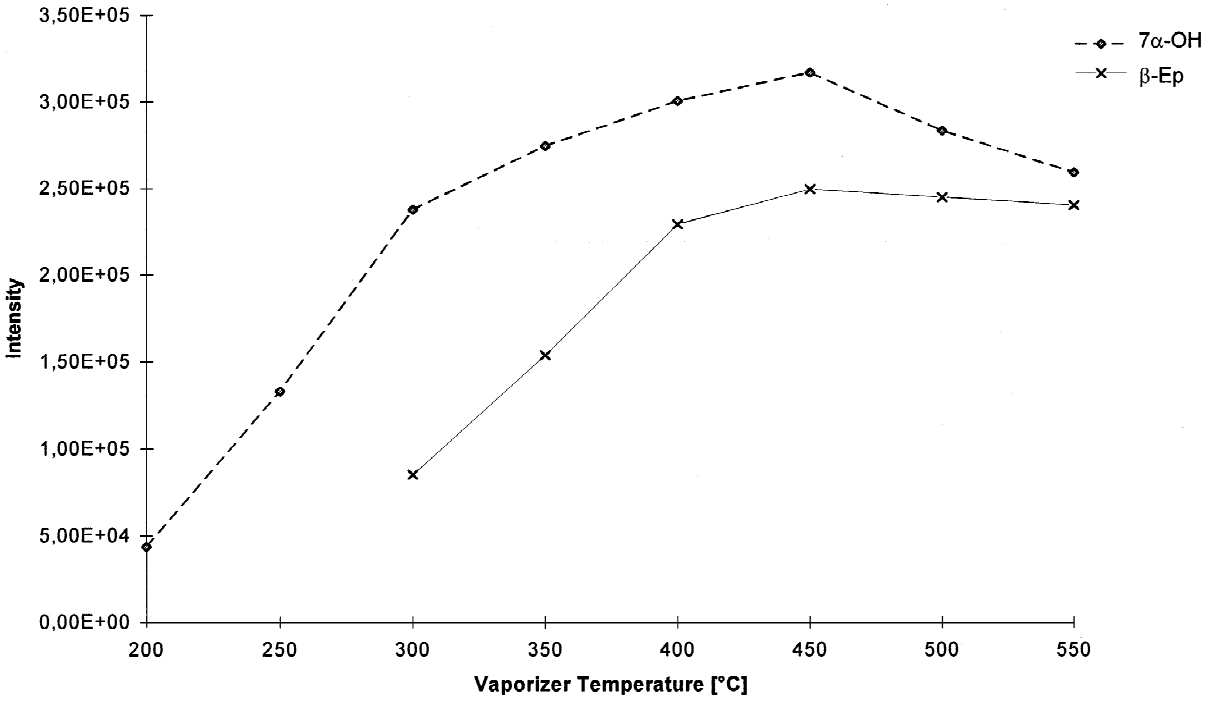

Fig. 2. Effect of APCI vaporiser temperature on the MS signal of 7α-hydroxycholesterol and 5,6β-epoxycholesterol.

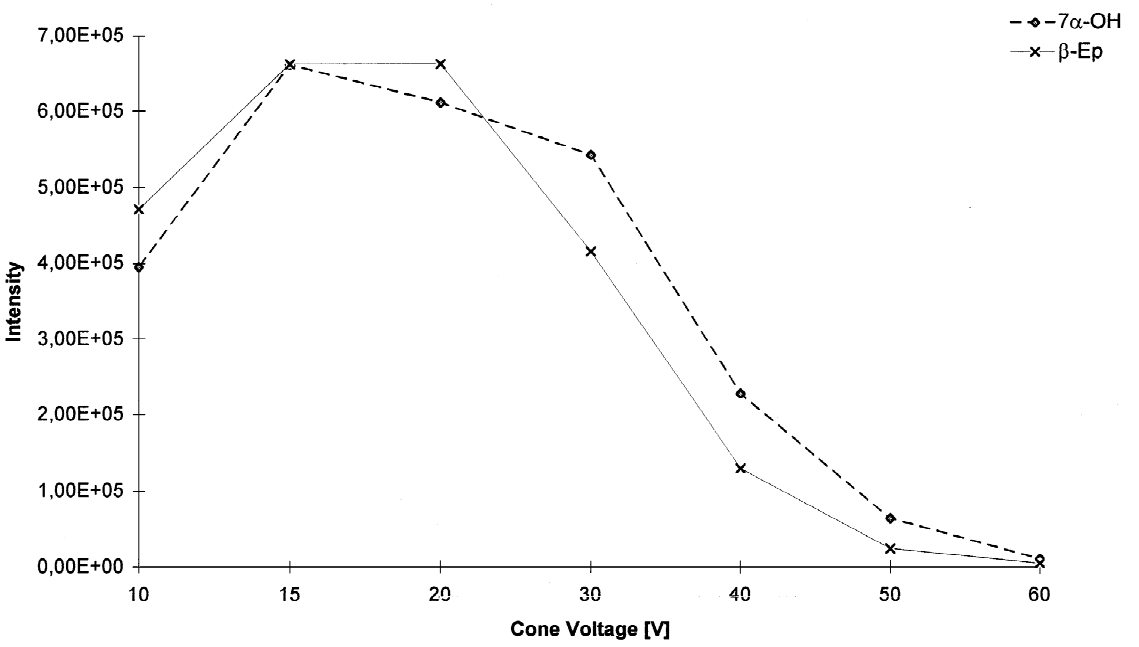

Fig. 3. The influence of cone voltage on signal intensity of 7α-hydroxycholesterol and 5,6β-epoxycholesterol.

a compromised cone voltage of 20 V for all tested compounds was used. The effect of cone voltage on the mass spectra of all COPs except 7-keto was very similar. In the case of 7-keto, the protonated molecule $[M+H]^+$ and the main fragment of $[M+H-H_2O]^+$ were stable even at higher cone voltages by using a vaporising temperature of 500°C, whereas an intensive fragmentation was observed for all other tested compounds with 40 V cone voltage. Generally, as expected, the intensity of protonated molecules and main fragments decreases by increasing the cone voltage.

3.2.3. Influence of source temperature

The effect of source temperature on signal intensity of COPs was investigated by varying the temperature between 100 and 200°C and keeping all other parameters constant. An improvement of the signal response could be achieved for all tested substances by increasing the source temperature. An optimum was found between 175 and 200°C, as can be seen in Fig. 4. The optimal temperature depends on the chemical properties of each individual compound as well as on the interface design and its geometry.

3.3. HPLC separation and detection by APCI-MS

As described before, 7α-hydroxycholesterol, 7β-hydroxycholesterol, 25-hydroxycholesterol, cholestan-3β,5α,6β-triol, 7-ketocholesterol, 5,6α-epoxycholesterol and 5,6β-epoxycholesterol show very similar fragmentation patterns. An appropriate separation of tested compounds was required for detection and determination of these compounds in SIM mode.

In preliminary experiments different stationary phases based on C_{18} with various mobile phase compositions were tested for separation of all seven cholesterol oxides. The variation of acetonitrile and methanol amounts as well as the use of water or ethanol in the mobile phase did not solve the coelution problems of tested COPs. The appropriate separation was achieved by using an Aquasil C_{18} column. The relatively hydrophilic nature of the applied material provides significantly more retention and selectivity for the separation of cholesterol oxides. The polar interactions between hydroxyl groups of COPs and the preserved hydrophilic sites on the surface of Aquasil column could be used to solve the major separation problems. The retention

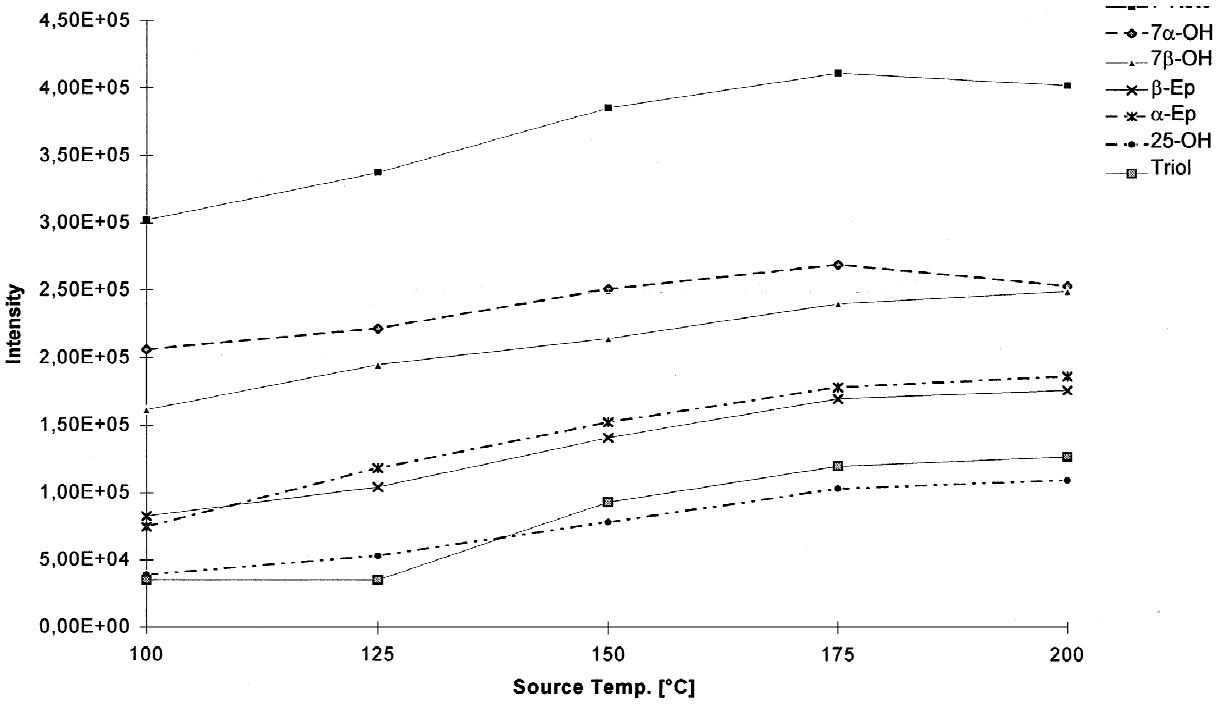

Fig. 4. Dependence of signal intensity of the COPs on the APCI source temperature.

times of tested compounds were highly reproducible as can be seen in Table 2. In the case of 25-hydroxycholesterol and 7β-hydroxycholesterol 20% overlapping of both compounds could be achieved. It was not possible to get a base peak separation between 7-ketocholesterol and 25-hydroxy-cholesterol. But using different fragment ions in SIM mode, the co-elution problem of both mentioned compounds could be solved as can be seen in Fig. 5.

Table 2
Reproducibility of retention times for tested cholesterol oxides

Analyte (n=6)	Retention time (min)	RSD[a] (%)
Triol	8.45	0.036
7β-OH	9.88	0.047
7β-OH	10.57	0.048
25-OH	10.92	0.071
7-Keto	11.10	0.052
β-EP	14.25	0.081
α-EP	16.59	0.089

[a] Relative standard deviation.

3.3.1. Quantitative analysis

Referring to the results of the scan experiments, base peak ions as well as main fragments for each cholesterol oxide were selected for quantitative analysis in SIM mode. Different foodstuffs such as egg powder, butter, butter oil as well as lard were analysed using the described method. The LC–AP-CI-MS chromatograms (in SIM mode) of blank and spiked butter samples and naturally contaminated egg powder are illustrated in Figs. 5–7.

For validation experiments in matrix only one butter sample with negligible amounts of COPs could be used for determination of detection limit and intra-assay precision. Generally we had problems by finding blank samples. All other analysed samples (butter, butter oil, lard and egg powder) were positive and showed high amounts of oxysterols.

The validation experiments were performed with pure standards and spiked butter. The limit of detection (S/N=3) for standards without matrix were lower than in matrix. As can be seen in Table 3, the

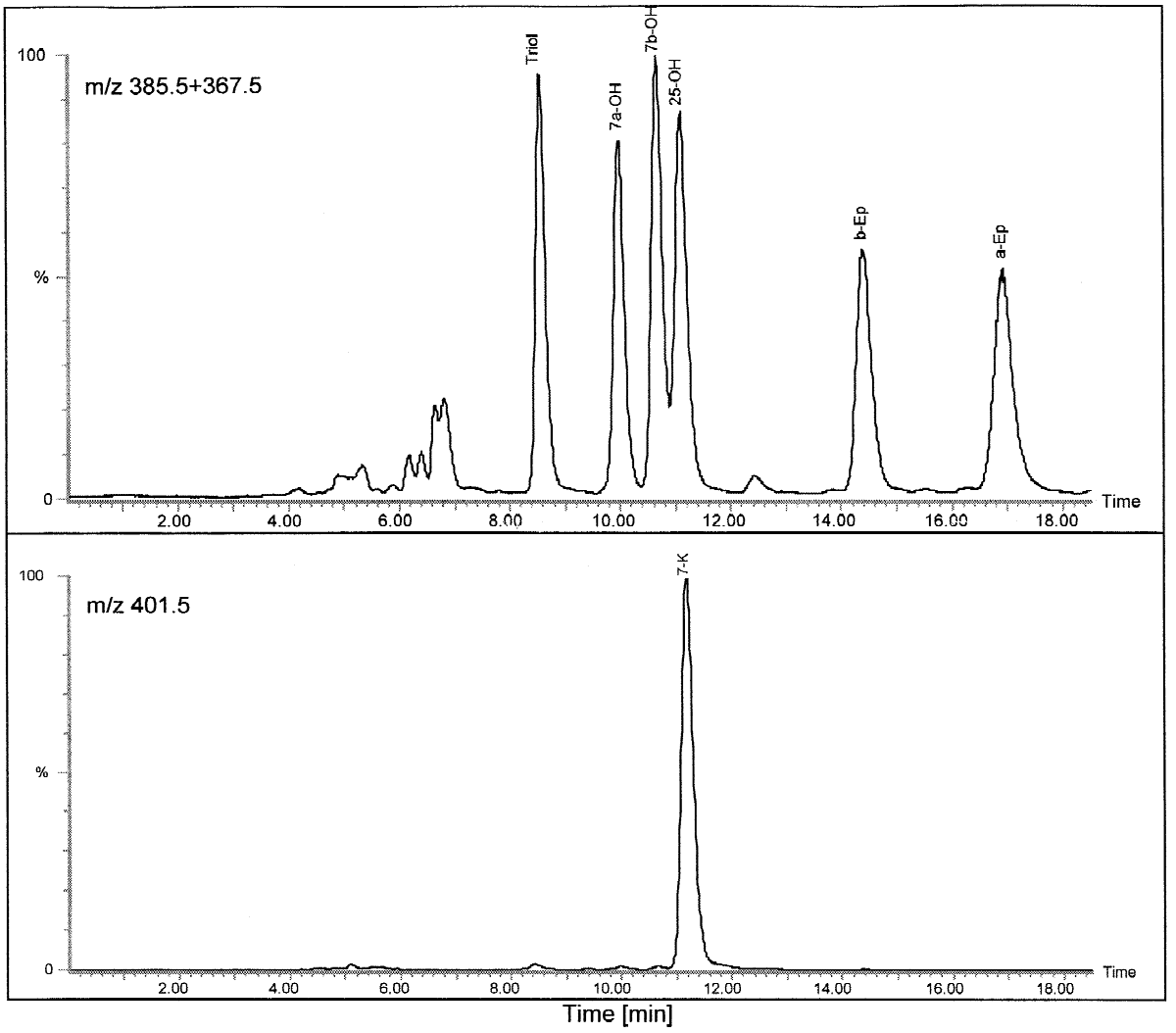

Fig. 5. SIM chromatograms (LC–APCI$^+$-MS) of a spiked butter sample containing 250 ng/g of each cholesterol oxide in different selected ions.

Table 3
Intra-, inter-assay precision expressed as relative standard deviation (RSD) and detection limits (LODs) in butter and pure standards for tested COPs

Analyte (n=6)	Intra-assay precision (RSD, %)		Inter-assay precision (RSD, %)		LOD	
	Standards 100 ng/ml	Butter 100 ng/g	Standards 100 ng/ml	Butter 100 ng/g	Standards ng/(abs.)	Butter ng/g (ng abs.)
7α-OH	2.4	4.4	9.5	15.9	0.5	15 (1.5)
7β-OH	2.3	3.3	10.0	15.5	0.5	15 (1.5)
25-OH	7.5	8.9	15.4	20.9	0.75	20 (2.0)
7-Keto	3.1	7.5	9.0	12.5	0.1	7 (0.7)
Triol	6.4	12.4	14.9	20.4	0.75	20 (2.0)
α-EP	0.8	5.3	8.2	16.7	0.5	15 (1.5)
β-EP	1.82	10.5	12.1	15.9	0.5	15 (1.5)

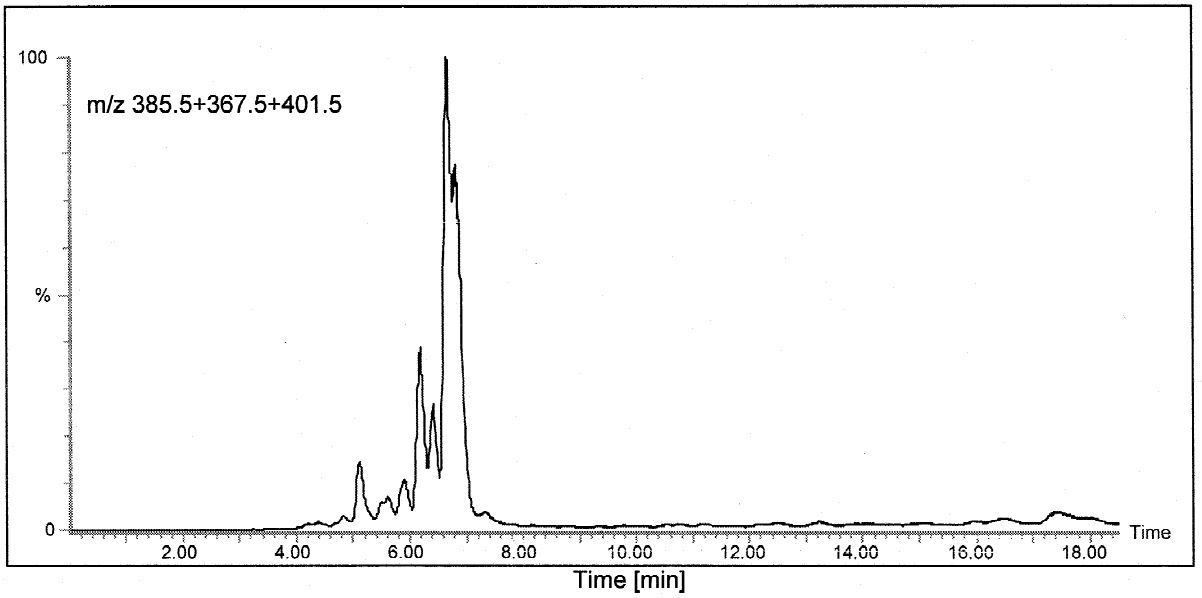

Fig. 6. LC–APCI$^+$-MS chromatogram of a blank butter extract in SIM mode.

best sensitivity in matrix as well as in standard solution could be observed for 7-ketocholesterol (7 ng/g). For 25-hydroxycholesterol as well as triol, the sensitivity of developed method with 20 ng/g was lower. For all other COPs similar detection limits could be observed (15 ng/g). Also the intra- and inter-assay precision (expressed as RSD) was determined in butter and pure standards, results are illustrated in Table 3. The reproducibility of the developed method in matrix, expressed as RSD, were generally lower than the pure standard solutions. The detection limits as well as intra-assay precisions, without matrix, are well comparable to those reported by Manini et al. [54].

Calibration curves were determined by external calibration in the concentration range between 0.02 and 10 μg/ml, which corresponds to a total amount of each COP from 1 to 500 ng injected. As can be seen in Table 4, excellent linearity has been observed for all tested compounds with correlation coefficients (r^2) of >0.997.

The recoveries (n=6) of developed LC–MS method for each cholesterol oxide were determined in four matrices (butter, butter oil, lard and egg powder). In egg powder compared to other samples, better recoveries for all tested cholesterol oxides could be observed (Table 5). Generally the recovery values in lard were lower than in other matrices. In

Table 4
Calibration graphs for linearity their error on slope and error on intercept as well as significance of intercept of tested COPs[a]

Analyte (n=6)	r^2	Calibration equation $y=ax+b$	Intercept error	Significance of intercept	Slope error
7α-OH	0.999	y=4217x+23 097	±3926	0.036	±42.5
7β-OH	0.998	y=3574x+11 174	±1676	0.186	±37.0
25-OH	0.998	y=4274x+6169	±1233	0.486	±41.1
7-Keto	0.999	y=7485x+6587	±922	0.245	±25.3
Triol	0.996	y=3090x−16 431	±2957	0.369	±83.8
α-EP	0.998	y=3848x+11 486	±1493	0.171	±36.6
β-EP	0.999	y=5153x+21 345	±3201	0.082	±50.6

[a] r^2 is the correlation coefficient, x is the injected concentration in ng and y is the peak area.

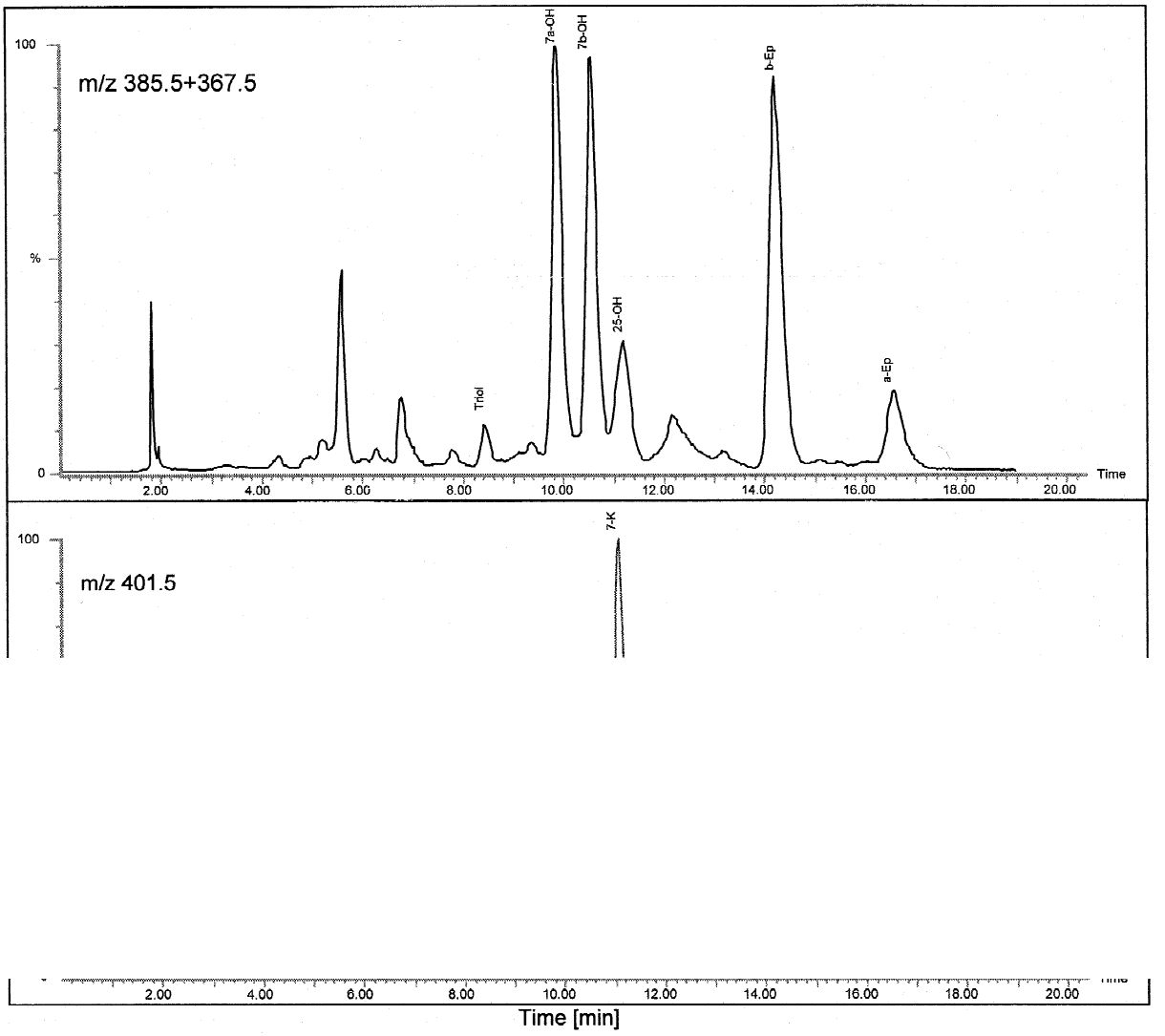

Fig. 7. SIM chromatograms (LC–APCI$^+$-MS) of naturally contaminated egg powder sample containing the following concentrations of COPs (ng/g): 7α-OH=472.1, 7β-OH=556.2, 25-OH=65.5, 7-keto=332.2, triol=58.4, α-EP=209.7, β-EP=546.6 in different selected ions. Peak A occurred only in egg powder samples and could not be identified.

butter oil and butter, the recovery of LC–MS method seems also to be good but in case of 6α-EP, β-EP and triol only poor recovery could be observed. For cholestan-3β,5α,6β-triol the recovery was lower in all four matrices, compared with other COPs.

To establish and test the performance of the developed LC–MS method, different fresh foods from the market (butter, butter oil, egg powder, lard) were purchased and the amount of COPs have been measured. In all these products cholesterol oxides could be detected, which revealed that the detection limit is sufficient for the tested COPs in different matrices. The results of analysis in different products were compared and are illustrated in Table 6. As can be seen, an increase of concentration of all tested cholesterol oxides in processed and heat treated foods could generally be observed, which corresponds to the results of other authors [22–27]. In egg

Table 5
Recovery of tested cholesterol oxides in different analysed foodstuffs

Analyte	Recovery (%)			
	Butter	Butter oil	Lard	Egg powder
7α-OH	92.8±3.0	97.6±2.5	86.9±2.2	97.9±2.6
7β-OH	89.5±2.5	95.8±2.7	89.8±3.8	105.3±4.9
25-OH	100.3±5.3	100.1±2.7	95.7±3.8	89.8±1.5
7-Keto	99.5±7.5	101.8±3.3	96.2±4.9	92.8±3.5
Triol	66.6±1.7	72.8±7.7	56.3±2.6	79.2±3.0
α-EP	69.8±0.8	78.8±5.6	74.6±3.5	100.6±2.6
β-EP	79.9±8.4	76.3±3.6	70.2±4.8	99.6±3.2

Table 6
Comparison of measured cholesterol oxide concentrations (ng/g) in different food samples (SIM ions were used for quantification)

Analyte	Concentration (ng/g)			
	Butter	Butter oil	Lard	Egg powder
7α-OH	34.8	96.0	91.8	472.1
7β-OH	16.6	45.5	93.4	556.2
25-OH	56.3	107.2	146.4	65.5
7-Keto	35.8	88.7	172.9	332.2
Triol	17.6	123.0	86.0	58.4
α-EP	18.4	213.2	43.2	209.7
β-EP	16.5	117.9	80.5	546.6

powder the measured concentration of some COPs were 10 times higher compared with other foodstuffs. The concentrations of 25-hydroxycholesterol and cholestan-3β,5α,6β-triol in butter oil and lard were higher. In butter, generally, the concentration of all measured COPs were the lowest one. The amount of 7-ketocholesterol was found to be higher than the other COPs in butter.

4. Conclusions

The use of MS as a specific detection method can improve the analytical possibilities and lead to higher sensitivity. The sensitivity of the described method is comparable to that of GC–MS. The developed method was found to be reasonably selective, sensitive and sufficiently robust for routine analysis in different foodstuffs. HPLC coupled on-line with MS seems to be the method of choice in the future for identification and quantification of toxic cholesterol oxides in biological matrices.

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