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Journal of Chromatography A, 724 (1996) 358–363

JOURNAL OF CHROMATOGRAPHY A

Short communication

Liquid chromatography–thermospray mass spectrometry of toremifene and its derivatives

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Received 20 March 1995; revised 12 September 1995; accepted 2 October 1995

Abstract

A high-performance liquid chromatographic–thermospray mass spectrometric method was developed for analysis of toremifene and eight of its derivatives, including some main metabolites. The compounds were chromatographed with an acetonitrile–ammonium acetate buffer eluent. Thermospray mass spectra of the compounds, showing abundant protonated molecular ions, were obtained with the exception of deaminohydroxy- and deaminocarboxy-toremifene, which apparently did not get protonated under these conditions. Selected-ion monitoring (SIM) of the protonated molecular ion of toremifene, the molecule of main interest in this series, was accomplished. According to the results, toremifene can be analyzed in the range from 0.01 μg/ml to 10 μg/ml, the limit of detection being 500 pg per injection. This method provides an effective means for purity assignation of forthcoming synthetic triphenylethylene derivatives. Liquid chromatography–thermospray mass spectrometry with selected-ion monitoring seems to be a sensitive and a selective quantitation method for toremifene.

Keywords: Liquid chromatography–mass spectrometry; Toremifene

1. Introduction

Tamoxifen is an established antiestrogenic agent, that has been used against breast cancer [1]. A structurally closely related triphenylethylene compound, toremifene, has been introduced recently, and it has proved to be an effective drug with long-lasting responses for the treatment of estrogen-receptor-positive advanced breast cancer [2,3]. Toremifene forms a variety of metabolites, some of them being pharmacologically active. Very few analytical methods for toremifene and its major metabolites have been described, all of them involving HPLC. The original method made use of fluorescence detection [4], the other two methods were based on UV detection [5] and on detection by mass spectrometry [6]. In this latter investigation the mass spectrometric detection system was equipped with an atmospheric pressure ionization (API) interface, and toremifene and four of its metabolites could be identified and quantitated from clinical samples.

HPLC–thermospray (TSP)-MS has proven useful in the confirmation of the identity of potential anticancer drugs and in the characterization of drug impurities as well as for studying possible degradation products of drugs and validation of HPLC–UV-based methods [7].

The aim of this investigation was to study the applicability of HPLC–TSP-MS for identification

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0021-9673/96/$15.00
SSDI 0021-9673(95)01126-9

and purity analyses of present and forthcoming triphenylethylene compounds. This study is a part of a research project that will concentrate on developing new drugs against cancer. Therefore an effective, specific and sensitive method is needed for quantitative analyses during drug development.

2. Experimental

2.1. Chemicals

Toremifene and its derivatives were synthesized by Orion-Farmos Pharmaceuticals (Turku, Finland). For the structural formulas of these compounds, see Table 1. The HPLC-grade acetonitrile was purchased from Rathburn (Walkerburn, UK), ammonium acetate and acetic acid from Merck (Darmstadt, Germany).

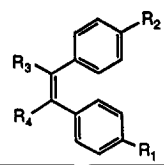
2.2. Liquid chromatography

The HPLC system consisted of an Applied Biosystems Model 400 solvent delivery system (Applied Biosystems) and a Rheodyne injector (Rheodyne, Cotati, CA, USA) with 50-μl loop. The column was a Supelco LC-8-DB, 5 μm particle size, 150×4.6 mm I.D. (Supelco, Bellefonte, PA, USA). The isocratic eluent consisted of 0.1 *M* ammonium acetate–acetonitrile (35:65). The pH of the buffer was adjusted to 6.5 with concentrated acetic acid. The flow-rate was set to 1.0 ml/min.

2.3. Mass spectrometry

The HPLC–MS system used was a VG thermospray–plasmaspray probe coupled to a VG Trio-2 quadrupole mass spectrometer (VG Masslab, Manchester, UK). The quantitative measurements were undertaken with the instrument in the thermospray mode. The ion-source temperature was 160°C, the vaporizer tip temperature being 160°C. The thermospray spectra were recorded for 5 μg of a compound chromatographed as described above. The selected-ion monitoring (SIM) of toremifene was based on the protonated molecular ion *m/z* 406. A four-point calibration curve of toremifene was created for the concentration range of 0.01–10 μg/ml, with linear regression being used for the calculation of the parameters of the curve.

Table 1
Structures of *cis*-triphenylethylene derivatives and their *m/z* values of protonated molecular ions $[M+H]^+$



Compound	R_1	R_2	R_3	R_4	$[M+H]^+$ (*m/z*)
Toremifene citrate	$OCH_2CH_2N(CH_3)_2$	H	CH_2CH_2Cl	C_6H_5	406
Deaminocarboxytoremifene	OCH_2COOH	H	CH_2CH_2Cl	C_6H_5	–[a]
Deaminohydroxytoremifene	OCH_2CH_2OH	H	CH_2CH_2Cl	C_6H_5	–[a]
FC-1158a citrate	$OCH_2CH_2N(CH_3)_2$	H	CH_2CH_2Br	C_6H_5	449
Demethyltoremifene citrate	$OCH_2CH_2NHCH_3$	H	CH_2CH_2Cl	C_6H_5	392
Didemethyltoremifene hydrochloride	$OCH_2CH_2NH_2$	H	CH_2CH_2Cl	C_6H_5	377
4-Hydroxytoremifene	$OCH_2CH_2N(CH_3)_2$	H	CH_2CH_2Cl	C_6H_5OH	421
FC-1530b citrate	$OCH_2CH_2N(CH_3)_2$	H	CH_2CH_3	Cyclopentyl	364
FC-1159a citrate	$OCH_2CH_2N(CH_3)_2$	H	CH_2CH_2I	C_6H_5	451[b]

[a] Not protonated.
[b] Obviously not the protonated molecular ion.

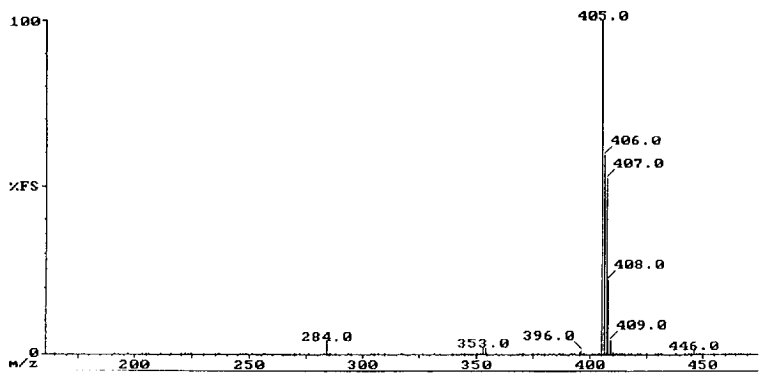


Fig. 1. Thermospray mass spectrum of toremifene. Conditions: 0.1 *M* ammonium acetate–acetonitrile (35:65); flow-rate 1 ml/min; source temperature 160°C; vaporizer temperature 160°C. A 5-μg mass of the compound was injected via a deactivated Supelco C_8 column (150×4.6 mm I.D., 5 μm). No discharge or filament was used.

3. Results

Protonated molecular ions could be produced for the toremifene and its derivatives described here, with exception of deaminohydroxy- and deaminocarboxy-toremifene. This may be due to the low proton affinity of these two compounds, both of them being metabolites of toremifene. The *m/z* values of the abundant protonated molecular ions for toremifene and its derivatives are presented in Table 1. The thermospray mass spectrum of toremifene shows the protonated molecular ion and also the ions due to the presence of the chlorine atom in the molecule (Fig. 1). The thermospray mass chromatogram of toremifene is shown in Fig. 2, indicating a retention time of 11.52 min in the liquid chromatographic system. In contrast to the deaminohydroxy derivative of toremifene, 4-hydroxytoremifene produced a protonated molecular ion *m/z* 421 in this HPLC–TSP-MS system, being attributable to satisfactory proton affinity in positive-ion TSP mode (Fig. 3A). An intense fragment peak of *m/z* 385 is a noticeable feature in this case. The TSP spectra of didemethyltoremifene (Fig. 3B) and FC-1158a are clearly distinguishable. The latter compound has one bromine atom in its structure, and therefore the

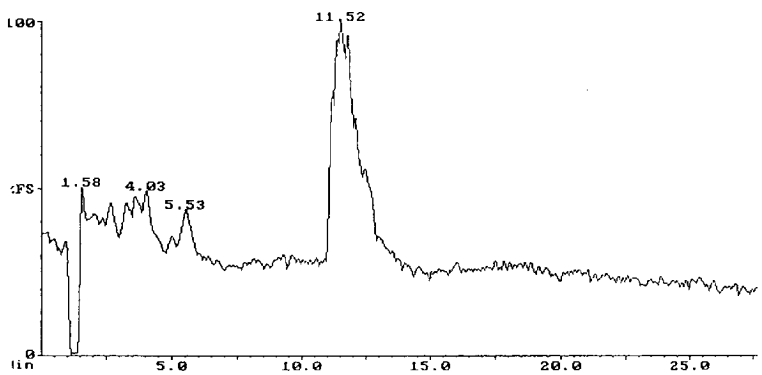


Fig. 2. Thermospray mass chromatogram of toremifene. Conditions as in Fig. 1.

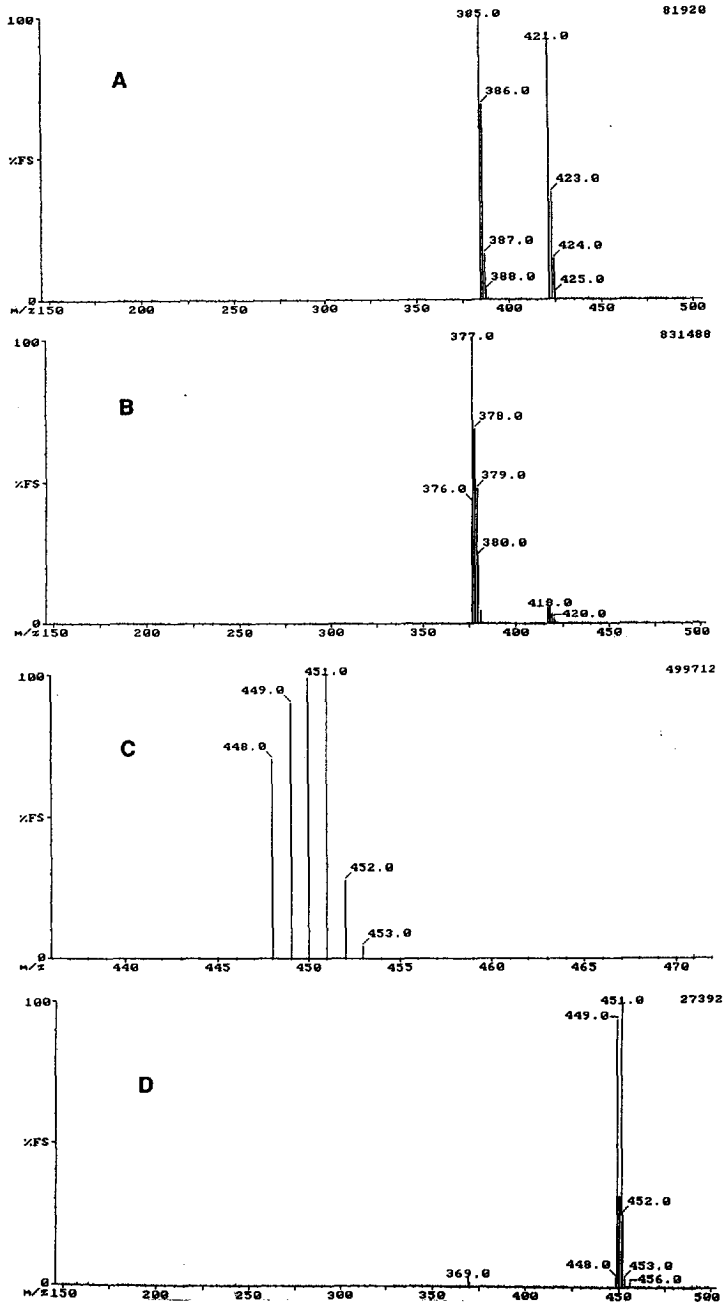


Fig. 3. Thermospray mass spectra of (A) 4-hydroxytoremifene; (B) didemethyltoremifene; (C) FC-1158a; (D) FC-1159a. For structures, see Table 1. Conditions as in Fig. 1.

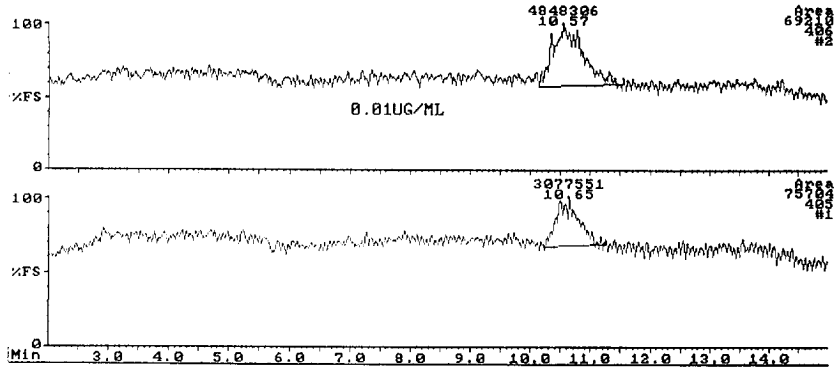


Fig. 4. Selected-ion chromatogram of toremifene (0.01 μg/ml in methanol) at m/z 406.

characteristic bromine isotope peaks can be seen (Fig. 3C). As for FC-1159a, having an iodine atom instead of chlorine or bromine, this compound seems not to exhibit the protonated molecular ion in the way that its bromine analogue (FC-1158a) does (Fig. 3D). SIM of toremifene (m/z 406) resulted in linearity from 500 pg to 500 ng per injection (r= 1.00), the limit of detection being 500 pg (0.01 μg/ml) of toremifene (Fig. 4).

4. Discussion

The analysis of toremifene and its metabolites has been described in only three publications [4–6]. All of these make use of a HPLC method, one of them being based on atmospheric pressure ionization (API) mass spectrometric detection for toremifene and four of its metabolites [6]. For toremifene and its derivatives, thermospray LC–MS methods have not been presented before. In our study, TSP-MS spectra could be produced for several metabolites and analogues of toremifene, including completely new compounds.

The method has good sensitivity for both toremifene and its derivatives, in the positive-ion thermospray mode. This can be explained in terms of the ability of the ammonium ion to protonate those solutes which have high proton affinities [7], with the exception of deaminohydroxy- and deaminocarboxy derivatives. These compounds do not possess proton accepting functional groups. The abundant fragment ion m/z 385 in the TSP spectrum of 4-hydroxytoremifene (Fig. 3A) is a different phenomenon when compared for instance with toremifene itself (Fig. 1) or other examined analogues. For the compound in question this kind of fragmentation has been explained in terms of loss of hydrochloride [M−36], the intensity of the fragment ion being quite low, however [6]. When the TSP spectra of FC-1158a (Fig. 3C) and FC-1159a (Fig. 3D) are compared, the latter compound is an exception, in that its probable protonated molecular ion is not distinguishable. The TSP mass spectrum of FC-1158a presents a characteristic bromine isotope pattern.

The compounds examined here seemed to be relatively pure according to their thermospray mass chromatograms and corresponding mass spectra. No significant impurities were found, which is consistent with the results obtained from the investigation of compounds using HPLC with diode-array detection (unpublished results).

Watanabe et al. have used 50–100 ng of compound per injection to obtain mass spectra of toremifene and its four expected metabolites [6]. The ultimate sensitivity for quantitation of toremifene or its metabolites was not clearly expressed in that investigation. In our HPLC–TSP-MS method, toremifene is measurable when 500 pg is injected (standard solution), but 5 μg of compound is needed for the recording of a TSP spectrum. In general, the atmospheric pressure ionization (API) technique is more sensitive than thermospray when combined with LC–MS [8].

This method provides an effective means for

purity assignation of forthcoming synthetic triphenylethylene derivatives. LC–TSP-MS with SIM seems to be a sensitive and selective quantitation method for toremifene. This method will now be further developed and applied in pharmacological studies, also for many new analogues. This is due to the fact that most of the toremifene derivatives investigated here produced abundant protonated molecular ions, being therefore good candidates for SIM measurements.

Acknowledgments

We are grateful to the Orion Farmos Corporation for providing toremifene and its derivatives. Mr. Jukka Knuutinen is acknowledged for his skilful technical assistance.

References

[1] P. Calabresi and P.A. Chabner, in A. Goodman-Gilman, T.W. Rall, A.S. Nies and P. Taylor (Editors), The Pharmacological Basis of Therapeutics, Pergamon Press, New York, 1990, pp. 1256–1257.
[2] L. Kangas, A.-L. Nieminen, G. Blanco, M. Grönroos, S. Kallio, A. Karjalainen, M. Perilä, M. Södervall and R. Toivola, Cancer Chemother. Pharmacol., 17 (1986) 109.
[3] R. Valavaara, S. Pyrhönen, M. Heikkinen, P. Rissanen, G. Blanco, E. Thölix, E. Nordman, P. Taskinen, L. Holsti and A. Hajba, Eur. J. Cancer Clin. Oncol., 24 (1988) 785.
[4] W.M. Holleran, S.A. Gharbo and M.W. DeGrigorio, Anal. Lett., 20 (1987) 871.
[5] L.K. Webster, N.A. Crinis, K.H. Stokes and J.F. Bishop, J. Chromatogr., 565 (1991) 482.
[6] N. Watanabe, T. Irie, M. Koyama and T. Tominaga, J. Chromatogr., 497 (1989) 169.
[7] R.W. Smith, C.E. Parker, D.M. Johnson and M.M. Bursey, J. Chromatogr., 394 (1987) 261.
[8] A.P. Bruins, J. Chromatogr., 554 (1991) 39.