Journal of Chromatography B, 878 (2010) 340–346

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

HPLC determination of novel dithiolethione containing drugs and its application for *in vivo* studies in rats

Daniela Giustarini[a], Elena Perrino[b], Valerio Tazzari[b], Ranieri Rossi[a,*]

[a] Department of Evolutionary Biology, Laboratory of Pharmacology and Toxicology, University of Siena, Via A. Moro 2, 53100 Siena, Italy
[b] Department of Pharmaceutical Sciences "Pietro Pratesi", University of Milan, Via Mangiagalli 25, Milan, Italy

ARTICLE INFO

Article history:
Received 19 August 2009
Accepted 30 November 2009
Available online 4 December 2009

Keywords:
Dithiolethione
Diclofenac
Valproic acid
Sildenafil
Aspirin

ABSTRACT

A panel of new drugs obtained by grafting a sulfurated moiety, i.e. 5-(4-hydroxyphenyl)-3*H*-1,2-dithiole-3-thione (ADTOH) onto existing drugs have been synthesized and their *in vivo* action is under preclinical evaluation. In the present paper we describe rapid HPLC methods to detect ADTOH derivatives of valproic acid (ACS2), sildenafil (ACS6), aspirin (ACS14) and diclofenac (ACS15) in plasma. These methods allow the simultaneous detection of the potential drugs and of ADTOH moiety. In the case of ACS14 the de-acetylated metabolite (ACS21) can also be concomitantly measured. The chromatographic separation was performed on a C18 column, applying a mobile phase consisting of a mixture of trifluoroacetic acid and acetonitrile. ADTOH, ACS6, ACS14, ACS21 were separated isocratically whereas ACS2 and ACS15 were separated applying gradient elution. The methods are precise and accurate, with a low quantification limit of 200 nM for ACS2, ACS15 and ACS21 or 100 nM for ADTOH, ACS6 and ACS14. The mean absolute recovery for all tested molecules was always found to be close to 100%. The methods are shown to be selective and linear in the range 0.2–50 μM and thus appear suitable for pharmacokinetic studies with ADTOH containing compounds, as indicated by exemplificative experiments performed with intravenous administration of the drugs to rats.

1. Introduction

Hydrogen sulfide (H_2S) is increasingly being recognized as a fundamental signaling molecule. It may modulate several physiological processes in the body including vasorelaxation, as does nitric oxide, another "gasotransmitter" [1,2]. H_2S was found to mediate the vasoactivity of garlic [3] and to attenuate myocardial ischemia–reperfusion injury by preservation of mitochondrial function in rats [4]. In addition, recent results have shown that H_2S and H_2S-releasing molecules are able to enhance intracellular antioxidant burden by means of several mechanisms, including glutathione increase and induction of the antioxidant and tissue protective protein heme oxygenase-1 [5–7].

The emerging data on the biological effects of H_2S have stimulated the development of sulfide-based therapeutics by means of H_2S-releasing compounds (sulfide donors) [8]. With this general approach in mind, new drugs obtained by grafting a sulfurated moiety, i.e. 5-(4-hydroxyphenyl)-3*H*-1,2-dithiole-3-thione (ADTOH) onto existing drugs have been synthesized (Fig. 1) and their *in vivo* action is under preclinical evaluation. ADTOH is known to be the main metabolite of anethole trithione (ADT, Sulfarlem®), a commercial drug used for its hepatoprotective properties and for the treatment of drug- and radiation-induced xerostomia, but also known to be endowed with cancer chemopreventive activity [9–11].

The effects of ADTOH derivatives of aspirin (ACS14), diclofenac (ACS15), valproic acid (ACS2) and sildenafil (ACS6), have been tested both in cell cultures and in rats. Results suggest that, while these drugs maintain the therapeutic capacities of the parent molecule, they have several advantages, e.g. they spare the gastric mucosa (ACS14, ACS15), increase histone deacetylase inhibition (ACS2), inhibit superoxide formation and up regulation of NADPH oxidase (ACS6) [12–16]. However, a method to detect these compounds is lacking so far.

The aim of this investigation was to develop rapid HPLC methods to detect both dithiolethione esterified molecules and their main metabolites (ADTOH and the de-acetylated metabolite of ACS14, ACS21) for the above-mentioned compounds. The methods were validated and applied to pharmacokinetic studies after *iv* administrations of the drugs to rats.

Abbreviations: ADTOH, 5-(4-hydroxyphenyl)-3*H*-1,2-dithiole-3-thione; ACN, acetonitrile; LLOQ, lower limit of quantification; TFA, trifluoroacetic acid; QC, quality controls; RE, relative error; RSD, relative standard deviation.

* Corresponding author. Tel.: +39 0577 234198; fax: +39 0577 234476.
E-mail addresses: giustarini@unisi.it (D. Giustarini), elena.perrino@fastwebnet.it (E. Perrino), valerio_tazzari@msn.com (V. Tazzari), ranieri@unisi.it (R. Rossi).

1570-0232/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2009.11.047

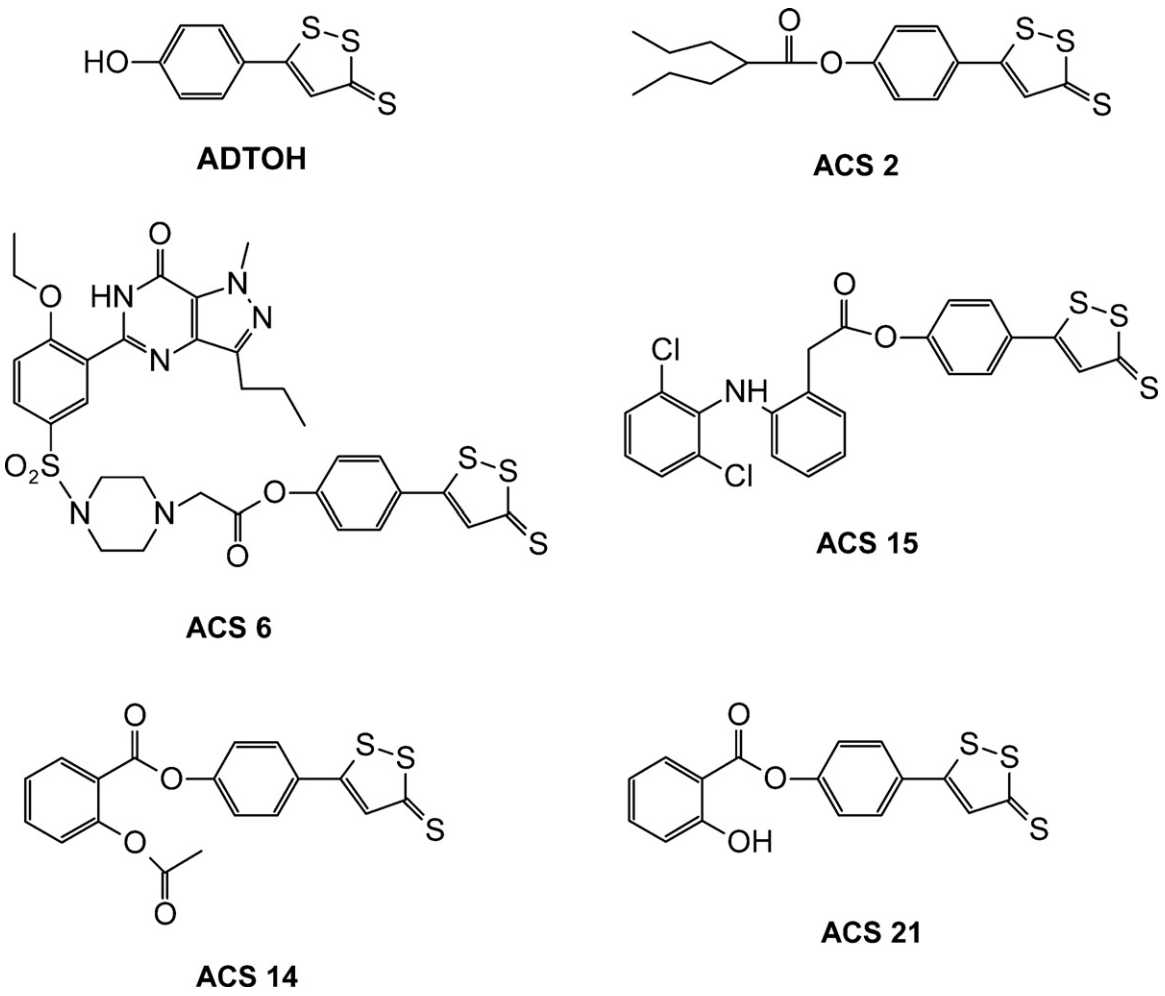

Fig. 1. Structural formulas of the studied compounds. The studied molecules are obtained by grafting a sulfurated moiety, i.e. 5-(4-hydroxyphenyl)-3*H*-1,2-dithiole-3-thione (ADTOH) onto existing drugs: valproic acid (ACS2), sildenafil (ACS6), aspirin (ACS14), diclofenac (ACS15). ACS21 is the de-acetylated metabolite of ACS14.

2. Materials and methods

2.1. Reagents

ADTOH, ACS2, ACS6, ACS14, ACS15 and ACS21 were prepared as previously described [12,13,15,17] by using chemicals and solvents of high purity grade purchased from Sigma–Aldrich, Milan, Italy. The identity and the purity of the products were confirmed by ^{1}H NMR, liquid chromatography mass spectrometry and elemental analysis [13]. Chromatographic purity was >98% for all products. HPLC grade acetonitrile (ACN) and trifluoroacetic acid (TFA) were from Mallinckrodt Baker (Milan, Italy). All other reagents were obtained from Sigma–Aldrich, Milan, Italy.

2.2. Animal treatments

Sprague–Dawley rats (350–400 g) were purchased from Charles River (Calco, Milan, Italy). A double valve (model 617, 20 mm × 20 mm, Danuso Instruments, Milano, Italy) was implanted in each animal: jugular and femoral veins were cannulated for either blood collection or drug administration, as previously described [18]. The valve was implanted under pentobarbital anesthesia (60 mg/kg) 2 days before the experiment. Animals were allowed to freely move and fed *ad libitum* before and during the experiments. Rats received administrations of drugs dissolved in 300 μl of polyethylene glycol (PEG) 400 *via* the cannula implanted in the femoral vein. Blood aliquots (~150 μl each) were collected through the valve connected to the jugular vein in tubes containing 5 μl K_3EDTA 50 mg/ml and immediately centrifuged at 12 000 × *g* for 15 s for plasma separation. All animal manipulations were made in accordance with the European Community guidelines for the use of laboratory animals. The experiments were authorized by the ethical committee of the University of Siena.

2.3. Spectroscopic analyses

All molecules were first dissolved in dimethyl sulfoxide (DMSO) at 5 mM final concentration and then diluted in (1:1, v/v) ACN:TFA 0.05% (v/v) to a final concentration of 50 μM. All spectra were recorded in the 200–700 nm wavelength range by means of a Jasco v550 UV–vis spectrophotometer.

Table 1
Run conditions applied to detect ACS2 and ACS15 by chromatography. Phase A: 0.05% (v/v) trifluoroacetic acid, phase B: acetonitrile.

Time	Phase A	Phase B	Flow (ml/min)
Start run	36%	64%	1.25
3.00 min	36%	64%	1.25
3.50 min	12%	88%	1.25
10.0 min	12%	88%	1.25

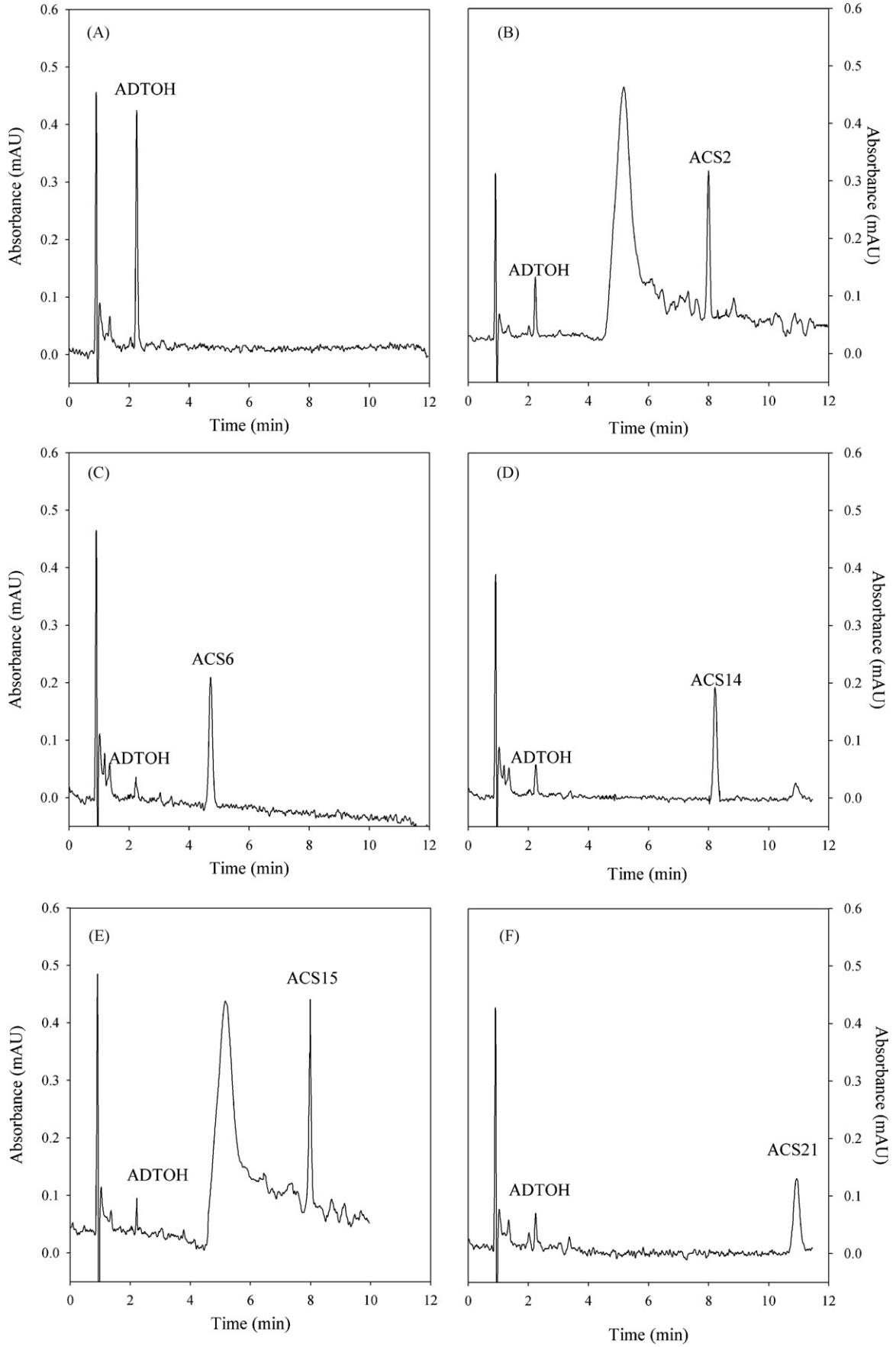

Fig. 2. Representative chromatograms of the studied compounds. Standard solutions of ADTOH (A), ACS2 (B), ACS6 (C), ACS14 (D), ACS15 (E), and ACS21 (F) were added to rat plasma samples (0.5 μM final concentration) and immediately analyzed by HPLC.

2.4. HPLC analyses

For the determination of salicylate, ADTOH, ACS2, ACS6, ACS14, ACS15, ACS21, 20 μl of plasma, obtained as above described, were added with 60 μl of ACN and vigorously shaken for 30 s. After protein discarding by centrifugation, 50 μl of the sample were added with 5 μl of 1% (v/v) TFA, loaded onto HPLC (Zorbax Eclipse XDB-C18 column, 4.6 mm × 150 mm, 5 μm, Agilent Technologies) and separated by the application of a mobile phase consisting of a mixture of 0.05% (v/v) TFA (phase A) and ACN (phase B).

ADTOH, ACS6, ACS14, ACS21 were separated isocratically with 64% phase B; ACS2 and ACS15 were separated applying gradient elution as shown in Table 1.

Mobile phase was delivered at 1.25 ml/min, column oven was set at 25 °C, the injection volume was 20 μl. An Agilent series 1100 HPLC equipped with diode array detector was used for all determinations. Analyses were recorded at 427 nm wavelength.

Salicylate, sildenafil and diclofenac were measured on the same plasma samples with HPLC using previously described methods [19–21]. Plasmatic levels of valproic acid were determined by immunoassay with a Modular system (Roche Diagnostics), using reagents from Dade Behring (Milan, Italy) according to manufacturer's instructions.

2.5. Method validation

The linearity was evaluated by preparing calibration standards in plasma at 0.1, 0.2, 0.5, 1, 2, 5, 10, 20 and 50 μM final concentration (from 5 mM stock solutions dissolved in DMSO) and assaying them in triplicate on 3 consecutive days. The lower limit of quantification (LLOQ) was calculated as the lowest (final) concentration of the added analyte that gives a peak with a signal to noise ratio greater than 10. The accuracy and precision were assessed by measuring quality control samples at three concentration levels (0.75, 7.5 and 15 μM) on 3 different days. The accuracy was expressed as relative error (RE, mean observed concentration − spiked concentration)/(spiked concentration) × 100% and the precision as relative standard deviation (RSD). In order to calculate the recoveries at two different concentrations (1 and 10 μM), the peak areas for plasma extracts were compared to the peak areas that were obtained by adding the quality controls to extracted blank plasma samples at the same final concentrations. The stability of the analytes was assessed at two concentrations (1 and 10 μM) in triplicate after storage of ACN extracted samples at −20 °C for 4 weeks.

3. Results and discussion

3.1. Spectroscopic analysis

In this study the following compounds, under evaluation for their therapeutic potential, have been analyzed: ACS2, ACS6, ACS14, ACS15. The dithiolethione moiety (ADTOH) and the de-acetylated metabolite of ACS14 (ACS21) have also been evaluated in our analyses. First of all, spectra of all these compounds were recorded in the 200–700 nm wavelength range. Typical absorbance peaks were found in the visible and UV regions for every analyzed molecule. In particular, all ADTOH containing molecules are characterized by typical absorbance peaks around 430 and 330 nm (not shown).

Table 2
Precision and accuracy values calculated for rat plasma samples added with different drugs at 200 nM (ACS2, ACS15, ACS21) or 100 nM (ADTOH, ACS6, ACS14) concentration (LLOQ).

Drug	RSD (%)	RE (%)
ADTOH	3.66 ± 0.58	−2.36 ± 0.33
ACS2	4.84 ± 0.33	−3.54 ± 0.22
ACS6	2.47 ± 0.49	2.59 ± 0.35
ACS14	2.88 ± 0.64	−0.354 ± 0.111
ACS15	3.81 ± 0.74	1.07 ± 0.21
ACS21	4.28 ± 0.36	4.39 ± 0.21

Precision is calculated as relative standard deviation (RSD), accuracy as relative error (RE). Data are the mean ± SD (n = 4).

Table 3
Recovery and stability of some ADTOH containing drugs added to plasma at two different concentrations.

Concentration (μM)	1	10
Recovery (%)		
ADTOH	98.4 ± 4.9	99.9 ± 0.5
ACS2	95.4 ± 2.7	97.8 ± 3.3
ACS6	101 ± 4.9	96 ± 3.0
ACS14	100 ± 2.7	96.6 ± 1.2
ACS15	94.2 ± 3.1	95.2 ± 3.1
ACS21	97.3 ± 0.7	103 ± 2.0
Stability (RE %)		
ADTOH	0.9	1.3
ACS2	0.6	−1.3
ACS6	−1.8	−1.9
ACS14	−2.1	−1.8
ACS15	−2.8	−3.3
ACS21	−0.5	−2.0

Values for recovery are expressed as percentage of the added quality control. Data are the mean ± SD (n = 3). Value for stability is expressed as relative error between the initial concentrations and the concentrations measured after storage at −20 °C for 4 weeks.

3.2. Selectivity

Chromatograms of rat plasma samples spiked with 0.5 μM (final concentration) of ADTOH containing molecules are shown in Fig. 2. The selectivity of the applied methods is evidenced by the fact that blank plasma samples (spiked only with an equal amount of DMSO) did not show any peaks with retention time similar to those given by other analytes (not shown). All ADTOH containing drugs

Table 4
Precision and accuracy values calculated for rat plasma samples added with the different compounds at three different concentrations.

Compound	RSD (%)		RE (%)	
	Intra-day	Inter-day	Intra-day	Inter-day
0.75 μM				
ADTOH	2.89 ± 0.36	2.55 ± 0.65	2.99 ± 0.65	−0.98 ± 0.47
ACS2	1.36 ± 0.58	2.47 ± 0.75	1.20 ± 0.32	−2.00 ± 0.58
ACS6	2.74 ± 0.74	3.20 ± 0.14	0.693 ± 0.03	1.08 ± 0.01
ACS14	2.69 ± 0.32	2.59 ± 0.74	−2.60 ± 0.11	−2.05 ± 0.14
ACS15	1.70 ± 0.01	2.47 ± 0.11	−1.96 ± 0.58	2.90 ± 0.24
ACS21	3.76 ± 0.24	3.69 ± 0.84	2.06 ± 0.32	1.35 ± 0.29
7.5 μM				
ADTOH	2.58 ± 0.24	3.01 ± 0.55	−0.965 ± 0.14	0.938 ± 0.415
ACS2	0.55 ± 0.48	2.48 ± 0.75	2.34 ± 0.43	−2.14 ± 0.68
ACS6	2.69 ± 0.39	2.77 ± 0.36	−2.41 ± 0.85	−1.11 ± 0.77
ACS14	2.15 ± 0.29	2.98 ± 0.47	3.28 ± 0.54	1.16 ± 0.45
ACS15	0.80 ± 0.14	1.92 ± 0.77	−2.00 ± 0.35	1.58 ± 0.38
ACS21	1.90 ± 0.44	3.89 ± 0.60	−2.30 ± 0.68	−1.82 ± 0.52
15 μM				
ADTOH	1.01 ± 0.03	2.20 ± 0.58	2.58 ± 0.59	−2.64 ± 0.73
ACS2	1.49 ± 0.14	3.65 ± 0.85	−1.39 ± 0.29	−2.08 ± 0.44
ACS6	2.39 ± 0.35	2.07 ± 0.69	−2.01 ± 0.63	−1.10 ± 0.89
ACS14	1.08 ± 0.12	2.90 ± 0.34	−1.08 ± 0.74	−2.54 ± 0.75
ACS15	1.25 ± 0.26	2.85 ± 0.74	−1.22 ± 0.41	1.99 ± 1.0
ACS21	2.09 ± 0.39	2.98 ± 0.40	0.32 ± 0.74	2.54 ± 0.62

Precision is calculated as relative standard deviation (RSD) and accuracy as relative error (RE). Data are the mean ± SD (n = 3).

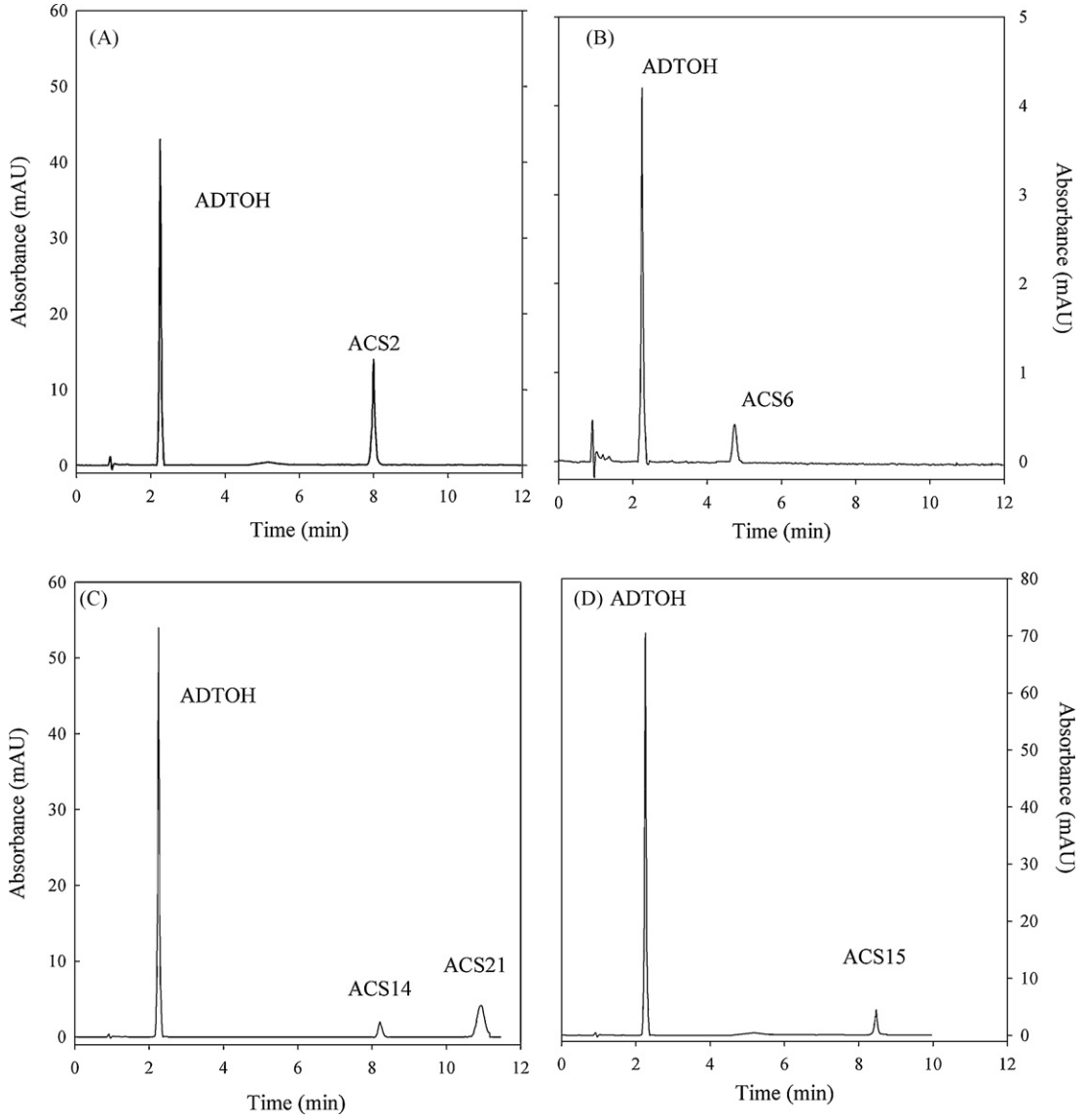

Fig. 3. Representative chromatograms of the studied drugs in *in vivo* experiments. Rats were *iv* administered with 15 mg/kg ACS2 (panel A), 2 mg/kg ACS6 (panel B), 15 mg/kg of ACS14 (panel C), 20 mg/kg ACS15 (panel D). Chromatograms were obtained by analyzing plasma after 10 min from the treatments.

are supposed to release ADTOH itself *in vivo* with typical kinetics, thus all analytical methods have been developed to be able to detect this molecule also. In confirmation of this, all the analyzed drugs show a small but evident peak corresponding to the rt of ADTOH (2.2 min). This suggests that: (i) some ADTOH is present as impurity and (ii) a minimal percentage of the drugs are hydrolyzed after its addition to plasma.

3.3. Linearity

The linear regression of the peak area *versus* concentration was fitted over the concentration range of 0.5–50 μM in rat plasma (data not shown). The concentrations of the calibration curve standards were chosen on the basis of the signals we obtained for different drugs after *in vivo* treatments in rats (see Section 3.6). In all cases a high linearity was observed and the obtained correlation coefficient *r* value was always >0.9990 (range 0.9993–0.9999). The LLOQ was found to be 200 nM for ACS2, ACS15 and ACS21 and 100 nM for ADTOH, ACS6 and ACS14. The accuracy and precision values obtained at these concentrations for each tested compound are reported in Table 2.

3.4. Recovery and stability

Data on recovery and stability were measured at 1 and 10 μM concentrations (Table 3). The mean absolute recovery for all tested molecules was found always to be close to 100% at both quality control levels.

To evaluate the stability, measurements of ACN extracts were repeated after their storage at −20 °C for 4 weeks. The relative error (RE) between the initial concentrations and the concentration after storage was less than 4% for all analyzed molecules, this indicates that samples are stable for at least 1 month under these conditions. Plasma samples stored at −20 °C showed a slow but significant decrease of the drug concentrations with formation of ADTOH over time (10–15% of the drugs were hydrolyzed in 4 weeks, not shown). Thus the best conditions we observed for storing these samples were in acidified acetonitrile (see Section 2).

3.5. Precision and accuracy

Precision and accuracy were calculated by adding each drug at three different concentrations to plasma: 0.75, 7.5 and 15 μM (final

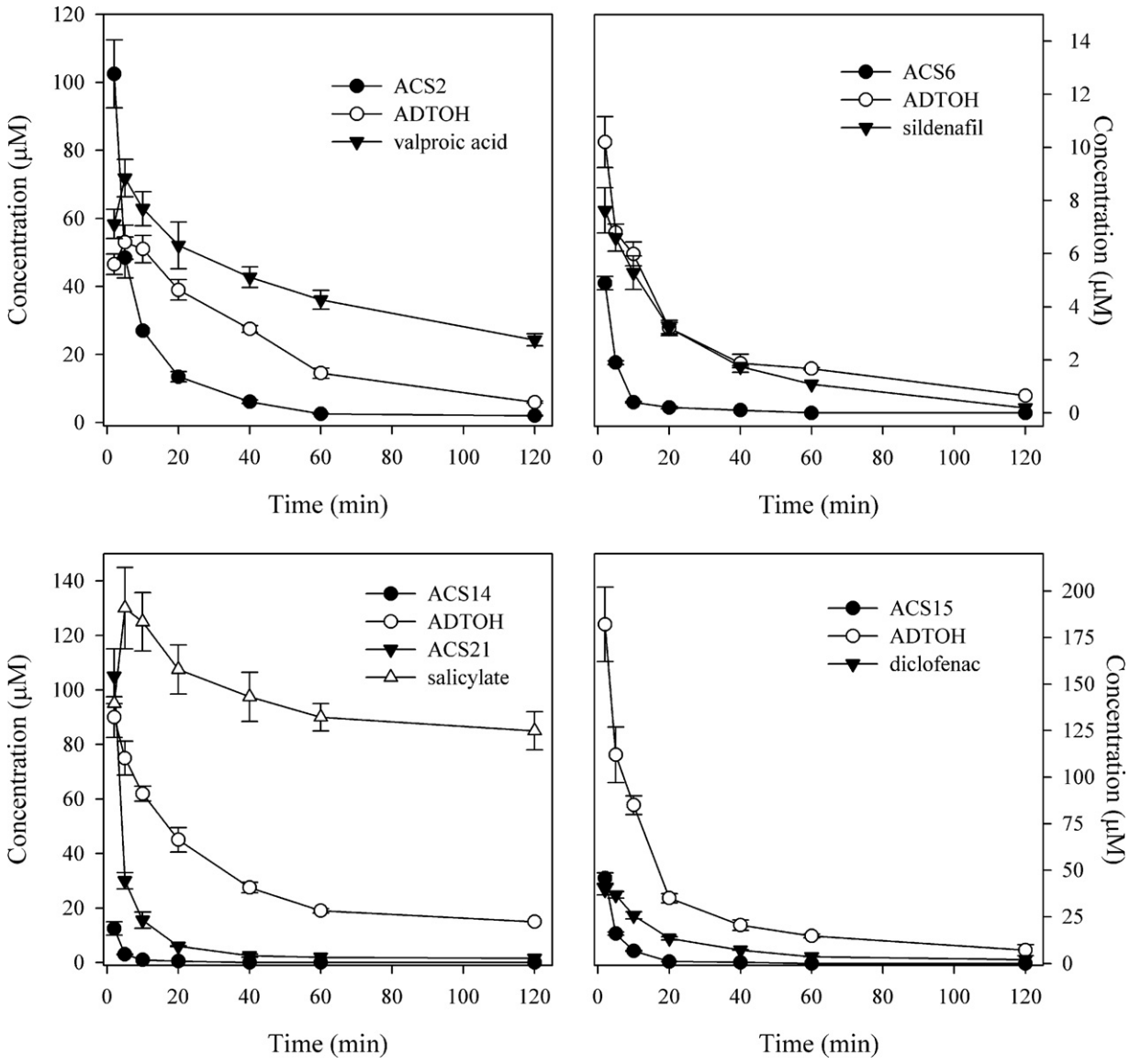

Fig. 4. Pharmacokinetic profile of the studied compounds. Rats were *iv* administered with 15 mg/kg ACS2, 2 mg/kg ACS6, 15 mg/kg ACS14, 20 mg/kg ACS15. At the indicated times plasma levels of the administered compounds; ADTOH and ACS21 (in rat treated with ACS14) were determined by the new HPLC methods. The parent compounds (valproic acid, sildenafil, salicylate, diclofenac) were also quantified in the same samples. Data are expressed as mean ± SD ($n = 4$). For those samples with a concentration exceeding calibration curve range chromatographic runs were repeated after appropriate dilution of the sample itself with a solution of 80% ACN.

concentrations). Intra-day and inter-day RE ranged from −2.6% to 4.3% and from −3.0% to 2.9%, respectively. Intra-day and inter-day RSD were both below 5%. Measured values are detailed in Table 4. These data suggest that the applied methods have high precision and accuracy.

3.6. Application of the methods

The ADTOH containing drugs were preliminarily tested for their stability both in PEG and aqueous buffer (Na^+/K^+ phosphate buffered solution, pH 7.4, PBS). Standard solutions (20 mg/ml) of ACS2, ACS6, ACS14, ACS15 were dissolved in PEG 400 and one aliquot was then diluted 1:100 in PBS. Samples (both in PEG and in PBS) were incubated at 37 °C and at 30 min time intervals aliquots were collected and analyzed. All the tested molecules were shown to be very stable in both matrices in that no evident decrease of their concentration occurred within 4 h (not shown).

The described assay methods were then applied to quantify ADTOH containing drugs and. the appearance of their main metabolites in plasma after their *iv* administration to rats. In particular, the experiments were performed with the aim of evaluating the kinetic of hydrolysis of the ester bond and the release of ADTOH from the studied drugs. An example of the obtained chromatograms for each drug after 10 min from treatments is shown in Fig. 3. All tested molecules were shown to be rapidly de-esterified (Fig. 4) as evidenced by the appearance of ADTOH within a few minutes from the treatment. Not surprisingly, ACS14 was rapidly de-acetylated forming ACS21 which in turn, was hydrolyzed yielding ADTOH. Hydrolysis of the ester bond and the appearance of ADTOH were paralleled by the increase in the parent molecule levels.

These experiments demonstrate that, once administered, all ADTOH containing drugs rapidly release both ADTOH and parent drugs. Kinetic of de-esterification was ACS15 > ACS6 > ACS14 > ACS2.

4. Conclusions

Two different HPLC methods were developed and validated for quantification of ADTOH containing drugs. The methods appear to be selective and accurate, with a quantification limit of either 100 or 200 nM. These LLOQ are close to those found by others for anethole trithione by using either HPLC or HPLC–MS/MS [22,23]. Our procedure has the advantage of being very rapid in that it does not require solid phase extraction or other time consuming extraction procedures: in fact supernatants from ACN treated plasma samples are directly injected onto HPLC. The methods appear suitable for pharmacokinetic studies with ADTOH containing drugs.

Acknowledgements

This work has been funded by PAR (Piano di Ateneo per la Ricerca, University of Siena) and by Fondazione Monte dei Paschi di Siena.

References

[1] R. Wang, FASEB J. 16 (2002) 1792.
[2] G. Yang, L. Wu, B. Jiang, W. Yang, J. Qi, K. Cao, Q. Meng, A.K. Mustafa, W. Mu, S. Zhang, S.H. Snyder, R. Wang, Science 322 (2008) 587.
[3] G.A. Benavides, G.L. Squadrito, R.W. Mills, H.D. Patel, T.S. Isbell, R.P. Patel, V.M. Darley-Usmar, J.E. Doeller, D.W. Kraus, Proc. Natl. Acad. Sci. U.S.A. 104 (2007) 17977.
[4] J.W. Elrod, J.W. Calvert, J. Morrison, J.E. Doeller, D.W. Kraus, L. Tao, X. Jiao, R. Scalia, L. Kiss, C. Szabò, H. Kimura, C.W. Chow, D.J. Lefer, Proc. Natl. Acad. Sci. U.S.A. 104 (2007) 15560.
[5] Z. Qingyou, D. Junbao, Z. Weijin, Y. Hui, T. Chaoshu, Z. Chunyu, Biochem. Biophys. Res. Commun. 317 (2004) 30.
[6] K. Erdmann, B.W. Cheung, S. Immenschuh, H. Schroder, Biochem. Biophys. Res. Commun. 368 (2008) 937.
[7] C. Szabó, Nat. Rev. Drug Discov. 6 (2007) 917.
[8] L. Li, P.K. Moore, Trends Pharmacol. Sci. 29 (2008) 84.
[9] A.N. Masoud, E. Bueding, J. Chromatogr. 276 (1983) 111.
[10] M.O. Christen, Methods Enzymol. 252 (1995) 316.
[11] R.A. Lubet, V.E. Steele, I. Eto, M.M. Juliana, G.J. Kelloff, C.J. Grubbs, Int. J. Cancer 72 (1997) 95.
[12] A. Sparatore, E. Perrino, V. Tazzari, D. Giustarini, R. Rossi, G. Rossoni, K. Erdman, H. Schröder, P. Del Soldato, Free Radic. Biol. Med. 46 (2009) 586.
[13] L. Li, G. Rossoni, A. Sparatore, L.C. Lee, P. Del Soldato, P.K. Moore, Free Radic. Biol. Med. 42 (2007) 706.
[14] E. Perrino, G. Cappelletti, V. Tazzari, E. Giavini, P. Del Soldato, A. Sparatore, Bioorg. Med. Chem. Lett. 18 (2008) 1893.
[15] S. Muzaffar, J.Y. Jeremy, A. Sparatore, P. Del Soldato, G.D. Angelini, N. Shukla, Br. J. Pharmacol. 155 (2008) 984.
[16] J.L. Wallace, G. Caliendo, V. Santagada, G. Cirino, S. Fiorucci, Gastroenterology 132 (2007) 261.
[17] J.S. Isenberg, Y. Jia, L. Field, L.A. Ridnour, A. Sparatore, P. Del Soldato, A.L. Sowers, G.C. Yeh, T.W. Moody, D.A. Wink, R. Ramchandran, D.D. Roberts, Br. J. Pharmacol. 151 (2007) 63.
[18] R. Rossi, A. Milzani, I. Dalle-Donne, F. Giannerini, D. Giustarini, L. Lusini, R. Colombo, P. Di Semplicio, J. Biol. Chem. 276 (2001) 7004.
[19] D. Yoshida, H. Todo, T. Hasegawa, K. Sugibayashi, Int. J. Pharm. 337 (2007) 142.
[20] H.S. Shin, S.K. Bae, M.G. Lee, Int. J. Pharm. 320 (2006) 64.
[21] G. Giagoudakis, S.L. Markantonis, J. Pharm. Biomed. Anal. 17 (1998) 897.
[22] Q. Jing, Y. Shen, F. Ren, J. Chen, Z. Jiang, B. Peng, Y. Leng, J. Dong, J. Pharm. Biomed. Anal. 42 (2006) 613.
[23] T. Li, Z. Zhang, H. Jiao, L. Zhang, Y. Tian, Y. Chen, X. Pang, J. Zhuang, Anal. Chim. Acta 594 (2007) 274.