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Studies of signal suppression in liquid chromatography–electrospray ionization mass spectrometry using volatile ion-pairing reagents

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Abstract

Volatile ion-pairing reagents are useful due to their compatibility with liquid chromatography–electrospray ionization (ESI) mass spectrometry. In this study trifluoroacetic acid, heptafluorobutanoic acid and perfluoroheptanoic acid were used as ion-pairing reagents. The signal intensities of eight amine analytes were measured in the presence of these fluorinated carboxylic acids and compared with the signal intensity when using an ion-pair free formic acid–ammonium formate buffer. It was shown that the ESI signal from most of the studied analytes decreased about 30–80% when the fluorinated carboxylic acids were added to the mobile phase at useful concentrations. The use of these acids in ion-pair chromatography was also compared to the more conventional sodium heptane sulphonate additive. It was found that the chromatographic performance was comparable. Finally, the long-term performance of the ESI interface and the chemical background caused by these fluorinated reagents were examined. No degradation of the ESI interface performance could be seen for over 24 h of continuous infusion.

Keywords: Electrospray ionization; Interfaces, LC–MS; Ion-pairing reagents; Amines

1. Introduction

LC–electrospray ionization (ESI)-MS is an important technique for the analysis of organic compounds due to its suitability for on-line separation and mass-detection. In the LC–ESI-MS analysis of highly polar ionic organic compounds it might, however, be challenging to find chromatographic separation conditions that are compatible with the electrospray ionisation process. It is well known that a high ionic strength of the mobile phase results in discharges and poor spray performance of the ESI interface [1]. Thus, it is preferred to use volatile buffer additives and to use them in as low concentration as possible to avoid contamination of the ESI interface and signal suppression of the analytes of interest. The maximum concentration of additives that can be used is dependent on the design and technical solutions of the ion-source of the mass spectrometric instrument, e.g. orthogonal electrospray instruments can often tolerate higher concentration of additives [2].

The separation of small polar compounds can be achieved by a retention mechanism based on ionic

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interaction. Ion-pairing in reversed-phase chromatography or ion-exchange chromatography can thus be used to obtain selective retention of polar compounds. However, with ion-exchange chromatography columns, buffers with high ionic strength have to be used to elute the analytes. The high ionic strength makes this kind of chromatography less compatible with the use of the ESI interface.

Ion-pair chromatography (IPC), on the other hand, uses surfactants as additives in the mobile phase to influence the chromatographic process. The concentration of ion-pairing reagents used in IPC is typically 1–3 m*M*. The additives are often long chain sulphonic acids with chains up to 12 carbon atoms. Sulphonic acids are, however, non-volatile and will quickly degrade the performance of the ESI interface. One useful solution to this problem has been the removal of the ion-paring reagent by an on-line procedure prior to MS [3]. This method requires the use of trapping columns that needs to be regenerated at regular intervals. Another, simpler approach is to use a more volatile ion-pairing reagent such as perfluorinated carboxylic acids [4–10]. However, there have only been few systematic studies on the effects these additives have on the behaviour of the electrospray process. It is known that the use of the LC buffer additive trifluoroacetic acid (TFA) in ESI can give lower response for an analyte, due to ion-pairing effects in the electrospray, than if the same sample was analysed with formic acid as an additive [11,12]. In this work, the effect on the ESI performance when using buffer additives like perfluorinated butanoic acid, perfluorinated heptanoic acid or TFA is compared to the use of formic acid in the buffer. Eight analytes with amine functionalities were chosen as test substances since analytes separated by ion-pair chromatography usually are amines. Furthermore, the long-term stability of the electrospray performance was studied over 24 h, using mobile phases containing the perfluorinated ion-pairing reagents. The chromatographic behaviour, concerning retention time, when using the perfluorinated ion-pairing reagents was compared to when sulphonic acids of equal length was used. The chemical background, i.e. the formation of adduct ions and the presence of molecules in the mobile phase causing a signal in the mass spectrum, was studied for perfluorinated ion-pair reagents together with the eight analytes.

2. Experimental

2.1. Materials

The following chemicals and solvents were used: methanol (Riedel-de Haën), nano-pure water (Elga maxima, Bucks, UK), ammonium formate (AMF) (BDH), formic acid (analytical-reagent grade) and trifluoroacetic acid (spectrometric quality) (both from Merck), heptafluorobutanoic acid, perfluoroheptanoic acid, tris(hydroxymethyl)aminomethane, harmine hydrochloride, DL-tyrosine and histidine (all from Sigma–Aldrich), *N*-methylspiperone and verapamil hydrochloride (RBI Research Chemicals, MA, USA), nicotine (Pharmacia, Sweden) and raclopride tartrate (Astra, Södertälje, Sweden).

2.2. Equipment

The mass spectrometer was a VG Quattro (Micromass, Manchester, UK). Pneumatically assisted electrospray was used with a cone voltage of 30 V, a capillary voltage of 3 kV and a cone temperature of 80°C. A Beckman 126 solvent delivery module (Beckman Instruments, CA, USA) was used at a flow-rate of 20 μl/min. Injections of the analyte solutions were made with a CMA autosampler (CMA/Microdialysis, Stockholm, Sweden). Injection volumes were 0.8 μl for the suppression studies. A 20-μl injection volume was used when scanning over several masses for the purpose of determining the chemical background.

The liquid chromatograph was a Jasco PU-980 (Tokyo, Japan) working at a constant pressure of 12 MPa (4 μl/min). Detection was made by a Jasco UV PU-975 detector equipped with a z-cell (Jasco flow cell) using wavelengths of 215 nm for the amino acids histidine and tyrosine, 259 nm for nicotine and 248 nm for harmine. The column was packed with Kromasil C_{18}, 5-μm particles (Eka Nobel, Nobel Industries, Bohus, Sweden) and the dimensions were 120 mm×200 μm. The mobile phase consisted of 10% methanol, 5 m*M* ammonium acetate 5 m*M*

acetic acid and 3 m*M* of the ion-pair reagent. A 0.5-m*M* solution (60 nl) of the amino acids, nicotine or harmine were injected through an internal Valco loop (Valco Instruments, Houston, TX, USA).

2.3. Flow injection procedures for the study of signal suppression and background

A buffer solution containing 5 m*M* ammonium formate, 5 m*M* formic acid and 10% (v/v) methanol was used. This solution was split into four bottles and to each of these bottles one of the following acids was added; 3 m*M* of formic acid, 3 m*M* TFA, 3 m*M* heptafluorobutanoic acid or 3 m*M* perfluoroheptanoic acid. 0.5 m*M* standard solutions of DL-tyrosine, *N*-methylspiperone, nicotine, histidine, verapamil, tris(hydroxymethyl)aminomethane, harmine and raclopride were prepared in 5 m*M* ammonium formate, 5 m*M* formic acid and 10% (V/V) methanol. Four sets of eight 5-μ*M* samples were made by diluting the standard solutions 100 times. For each of the four sets of samples one of the previously prepared buffers was used for dilution giving totally 32 combinations of samples and buffers.

Ten 0.8-μl injections from each of these samples were made into the mass spectrometer and the single ion trace corresponding to the (M+1) mass was recorded. All samples were injected into a stream of the corresponding buffer used for the sample dilution. Before changing to the next buffer system a larger injection volume, 20 μl, was used and each sample was injected once with scans between *m*/*z* 110 and *m*/*z* 800 acquired to determine the chemical background.

2.4. Long-term stability study

A buffer was made containing 5 m*M* ammonium formate and 5 m*M* formic acid. This buffer was divided in three parts and to each part one of the following acids was added; 3 m*M* formic acid, pentafluorobutanoic acid or perfluoroheptanoic acid. Samples of 5 μ*M* verapamil were made up with each buffer. Flow-injection studies were made with an injection volume of 0.8 μl and with a flow of 20 μl/min. Injections were made every 30 min during a period of 24 h. This was made once for each buffer system. The single ion trace at *m*/*z* 456 (verapamil, M+1) was measured by MS.

3. Results and discussion

3.1. Signal suppression

The effect on the signal of small polar analytes in the presence of different buffer additives was tested. Eight typical analytes, each containing at least one amine functionality, as seen in Fig. 1, was thus used to determine the effect on signal intensity of the MS. The signal intensities of these analytes were determined in each of four different buffer systems. All buffers contained an AMF–formic acid buffer with added perfluorinated carboxylic acid or formic acid. The pH was allowed to vary between 3.4 and 3.7 and the ionic strengths were held constant in the buffers used. All the voltages used in the ESI were also held constant throughout the experiments. The peak areas obtained for each analyte in the neat formic acid–AMF buffer are summarised in the first column in Table 1. The second column shows the relative response obtained when a short fluorinated carboxylic acid, TFA, was added to the buffer.

The following two columns show the effect of longer fluorinated acids additives to the buffer. For the first five analytes in Table 1, the original signal intensities were relatively high and significant signal suppression was noted when adding TFA and the other fluorinated acids. The peak area when using TFA in the buffer was thus only between 45 and 60% of the peak area obtained when only formic acid was used. When adding the longer chain fluorinated carboxylic acids to the mobile phase, the response decreased even further to between 15 and 30% of the signal intensity obtained for neat formic acid buffer. Tris and the two amino acids tyrosine and histidine gave, on the other hand, much lower signal intensity also with formic acid buffer. As seen from the partition coefficients listed in Table 2, these analytes have a strong hydrophilic character. It is thus expected that these polar analytes prefer the interior of the ESI droplet, which would lead to a lower presence and thereby a lower tendency to

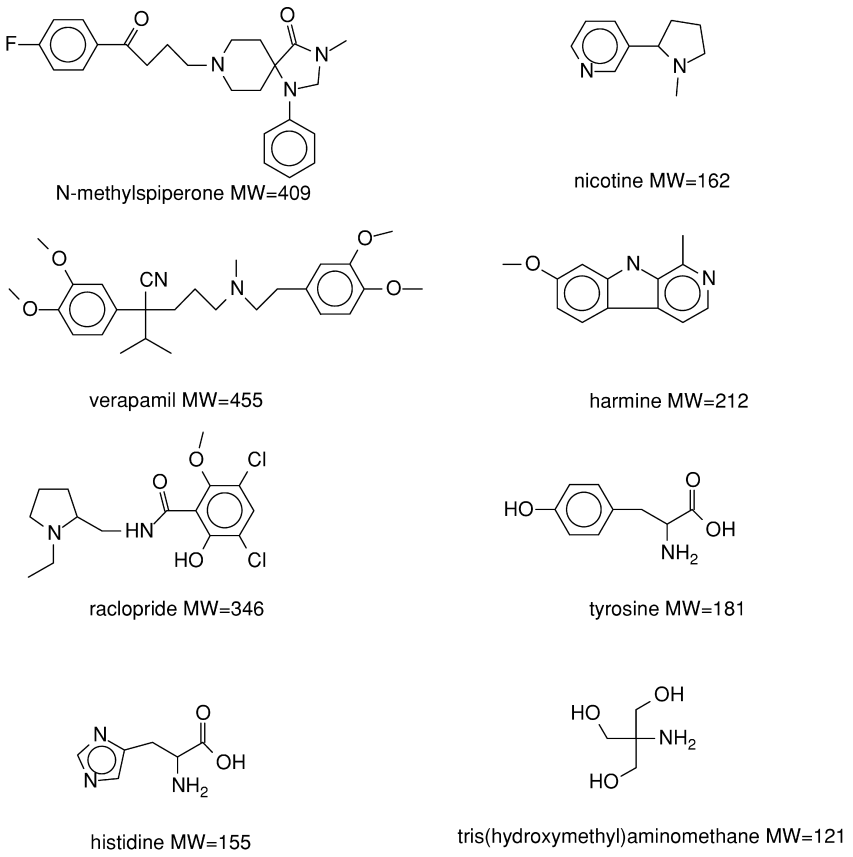

Fig. 1. Chemical structures and molecular masses (MW) of eight selected amine analytes used in this study.

Table 1
Effects of different buffer additives on positive ion ESI sensitivities for selected amine analytes

	Buffer without ion-pair	Buffer with fluorinated additives		
	Formic acid (counts×10^6)[a]	TFA (%)[b]	Heptafluorobutanoic acid (%)[b]	Perfluoroheptanoic acid (%)[b]
N-Methylspiperone	1.70	60	30	15
Nicotine	0.98	50	20	31
Verapamil	3.18	44	25	20
Harmine	1.68	61	26	26
Raclopride	1.11	57	26	19
Tyrosine	0.008	126	102	102
Histidine	0.023	103	97	149
Tris	0.045	90	68	87

[a] Response areas obtained with 3 m*M* formic acid added to the 5 m*M* AMF–5 m*M* formic buffer.
[b] Response areas obtained when adding 3 m*M* of the fluorinated carboxylic acids to the 5 m*M* AMF–5 m*M* formic buffer. The areas are given as percent of the response areas obtained with the formic acid additive.

Table 2
Partition coefficients (log *p*) and pK_a of selected amine analytes

	Log *p*	pK_a
N-Methylspiperone	2.92[b]	–
Nicotine	0.93[a]	2.93[c], 8.18[c]
Verapamil	3.74[a]	8.7[c]
Harmine	0.9[a]	6.6[c], 12.5
Raclopride	1.31[a]	9.21[c]
Tyrosine	−1.94[a]	2.2, 9.07[c], 10.09
Histidine	−2.93[a]	1.8, 6.0[c], 9.2[c]
Tris	−1.56[b]	8.2[c]

[a] Data from Beilstein.
[b] Calculated from http://esc.syrres.com/interkow/kowdemo.htm.
[c] The pK_a is given for the acid form of the amines.

evaporate from the droplet surface and a lower response in ESI is therefore not surprising [13,14]. In agreement with this discussion, verapamil with the highest partition coefficient (Table 2) would prefer to be at the surface of the ESI droplets and subsequently it also gave the highest response in the ESI–MS analysis. In this study the most polar analytes also showed hardly no change in signal intensity when using the fluorinated acids instead of formic acid in the buffer, see Table 1. This suggests that they do not participate in ion-pairing in the ESI process. The more nonpolar analytes, with the higher partition coefficients, also seem to have a greater tendency for ion-pair formation in the ESI as the ion signal clearly decreases with the longer ion-pairing reagent.

The difference in analyte response caused by the fluorinated carboxylic acid with longer hydrophobic chain, compared to TFA, can have several explanations [1,15]. One reason could be variation in volatility of the different ion-pairs, with the shorter ones being more volatile, which would have an impact on the ionisation efficiency of the analytes in the electrospray. Other important factors are surfactant properties, such as surface activity, which influences the ionisation efficiency of the droplets formed in the electrospray process [11].

As observed by others [16], there seemed to be no correlation between the analytes' pK_a values in Table 2 and their ESI responses, seen in Table 1.

It should also be noted that micro flow-rates are used in this study. Although there is a trend today to make ESI interfaces handle higher flow-rates; it should be remembered that lower flow-rates always are preferred for optimal ionization from a liquid flow. While a split flow is often used in LC–MS, it is of interest to gain enhanced sensitivity in combination with separation using micro LC if robust systems and methods can be developed.

3.2. Chromatography

Generally, the length of the carbon chain of sulphonic acid ion-pairing reagents is correlated to the effect of the additive. The longest sulphonic acid commonly used is sodium dodecyl sulphate (SDS). Chromatography with SDS as an ion-pair reagent requires several hours column equilibration before repeatable separations can be achieved. For this reason shorter sulphonic acids may be used, e.g. heptanesulphonic acid, which gives good retention characteristics with shorter equilibration times.

Volatile ion-pairing reagents have not been used in ion-pair chromatography to the same extent as their non-volatile counterpart sulphonic acids. Heptafluorobutanoic acid and perfluoroheptanoic acid as ion-pairing reagents in ion-pair chromatography have, however, been demonstrated to work well in the analysis of amines [5].

In this study, four analytes, i.e. tyrosine, histidine, harmine and nicotine were analysed by IPC using both the volatile ion-pairing reagents heptafluorobutanoic acid, perfluoroheptanoic acid and the non-volatile heptanesulphonic acid. The retention factors obtained for each analyte with the different buffer systems are summarised in Table 3.

From the results shown in Table 3, it can be concluded that the fluorinated ion-pair reagents may be used instead of heptanesulphonic acid. There is, however, no straight correlation between the fluorinated carboxylic acids and the sulphonic acids regarding the retention factor, k'. The pH in the mobile phases is buffered to 3.5 so that the ion-pairing reagents are fully dissociated (p$K_a \approx 0$). For the histidine and tyrosine, the heptane sulphonic acid and the heptafluorobutanoic acid gives the same effect and the perfluoroheptanoic acids gave a higher k'. Nicotine did not elute within the analysis time of 40 min when perfluoroheptanoic acid was used in the mobile phase. Harmine did not elute within 40 min when heptane sulphonic acid was used as ion-pairing

Table 3
Retention factors (k') for three mobile phases containing different ion-pairing reagents, heptafluorobutanoic acid, perfluoroheptanoic acid and heptanesulphonic acid

	Retention factor (k')		
	Heptafluorobutanoic acid	Perfluoroheptanoic acid	Heptanesulphonic acid
Tyrosine	0.6	3.4	4.7
Histidine	1.5	19	5.9
Harmine	18	38	–
Nicotine	4.8	–	22

reagent. Attempts have been made to predict electrospray response from k' by Chech et al. [17]. They suggested that analytes with a more hydrophobic character, i.e. with a longer retention time, have a higher ESI response. The amino acids in this study are very polar with a low k' and consequently they show a low ESI response as may be seen in Table 1. This is explained by partitioning within the ESI droplet, the more polar analytes have a greater tendency for the droplet interior and are therefore ''shielded''.

The amino acids were injected three times to check the repeatability of the peak areas. Heptanesulphonic acid and heptafluorobutanoic acid gave narrow peaks and a good repeatability, RSD: 5–9%, while perfluoroheptanoic acid gave worse repeatability, RSD: 12–13%, and broader peaks suggesting adsorption effects in the column.

It can be concluded that with the conditions used in this study, fluorinated carboxylic acids may replace sulphonic acids as ion-pairing reagents when analysing amines. The efficiency for these four analytes is comparable for heptanesulphonic acid and heptafluorobutanoic acid.

3.3. Long-term stability

One problem when using sulphonic acids as ion-pairing reagent is that they will leave solid residues inside the ESI interface [3]. These depositions will lead to unstable spray performance and loss of signal. To investigate if the same problem exist with perfluorinated carboxylic acids, a mobile phase with one of these acids was sprayed for approximately 24 h. Every 30 min, an injection of verapamil was made and the single ion trace of m/z 465 was recorded. The collected MS data from injections of verapamil during a 24-h period are summarised in Fig. 2. The response from the mass spectrometer was more or less unchanged during the 24 h period for all three buffer systems: formic acid–AMF, formic acid–AMF–heptafluorobutanoic acid and formic acid–AMF–perfluoroheptanoic acid. The results indicated that there were no major problems with instabilities due to deposition of non-volatile material from the ion-paring reagents on the electrospray cone or high-voltage lens.

3.4. Formation of adducts in the MS spectra

The use of sulphonic acids as ion-pair reagents will not only result in a quicker contamination of an electrospray interface but also cause major adduct formation of the analyte of interest, primarily with sodium ions. The source of sodium in the mobile phase is mainly from the sulphonic acids that are used in form of sodium salts. The fluorinated carboxylic acids on the other hand are available as the free acid and consequently no sodium adducts were observed in those spectra.

4. Summary

Perfluoroheptanoic acid and heptafluorobutanoic acid works well as ion-pairing reagents in IPC. It was shown that a buffer containing these fluorinated acids at 3 mM concentration could be used with LC–ESI-MS for more than 24 h without any degradation of the ESI performance. However, the peak areas obtained when analysing the amine analytes were significantly lower when exchanging a part of the formic acid in the buffer to a fluorinated carboxylic acid. The response when using TFA was

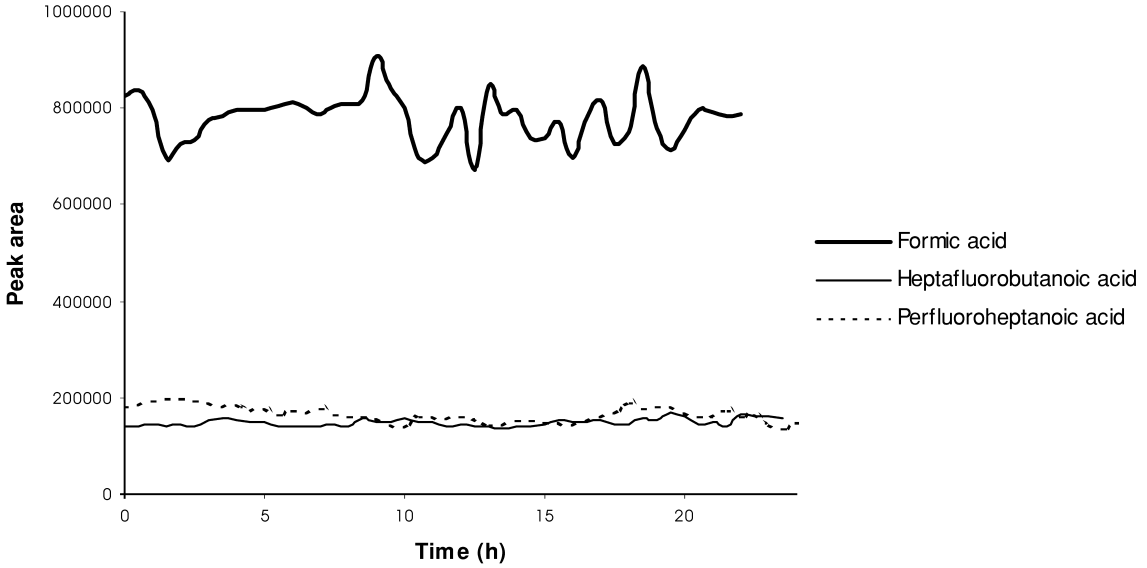

Fig. 2. Response areas for repeated injections of verapamil, m/z 456, during approximately 24 h with different carboxylic acids in the mobile phase.

45–60% of the response obtained when having formic acid in the buffer. When using perfluoroheptanoic acid and heptafluorobutanoic acid in the buffer the response for the amine analytes decreased to between 15 and 30% of the value obtained with formic acid in the buffer.

References

[1] M.G. Ikonomou, A.T. Blades, P. Kebarle, Anal. Chem. 62 (1990) 957.
[2] K. Hiraoka, H. Fukasawa, F. Matsushita, K. Aizawa, Rapid Commun. Mass Spectrom. 9 (1995) 1349.
[3] B.H. Forngren, J. Samskog, S.Å. Gustavsson, N. Tyrefors, K.E. Markides, B. Långström, J. Chromatogr. A 854 (1999) 155.
[4] P. Chaimbault, K. Petritis, C. Elfakir, M. Dreux, J. Chromatogr. A 855 (1999) 191.
[5] P. Chaimbault, K. Petritis, C. Elfakir, M. Dreux, J. Chromatogr. A 870 (2000) 245.
[6] J. Keever, R.D. Voyksner, K.L. Tyczkowska, J. Chromatogr. A 794 (1998) 57.
[7] J.K. Roberts, M.J. Hughes, J. Chromatogr. A 828 (1998) 297.
[8] R. Castro, E. Moyano, M.T. Galceran, J. Chromatogr. A 869 (2000) 441.
[9] Y.X. Zhu, P.S.H. Wong, M. Cregor, J.F. Gitzen, L.A. Coury, P.T. Kissinger, Rapid Commun. Mass Spectrom. 14 (2000) 1695.
[10] K.N. Petritis, P. Chaimbault, C. Elfakir, M. Dreux, J. Chromatogr. A 833 (1999) 147.
[11] A. Apffel, S. Fischer, G. Goldberg, P.C. Goodley, F.E. Kuhlmann, J. Chromatogr. A 712 (1995) 177.
[12] F.E. Kuhlmann, A. Apffel, S.M. Fischer, G. Goldberg, P.C. Goodley, J. Am. Soc. Mass Spectrom. 6 (1995) 1221.
[13] C.G. Enke, Anal. Chem. 69 (1997) 4885.
[14] J.V. Iribarne, P.J. Dziedzic, B.A. Thomson, Int. J. Mass Spectrom. Ion Phys. 50 (1983) 331.
[15] S.K. Chowdhury, B.T. Chait, Anal. Chem. 63 (1991) 1660.
[16] K. Hiraoka, K. Murata, I. Kudaka, J. Mass Spectrom. Soc. Jpn. 43 (1995) 127.
[17] N.B. Cech, J.R. Krone, C.G. Enke, Anal. Chem. 73 (2001) 208.