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Fast and direct recognition of the active ingredients in tablets using hot cell membrane inlet mass spectrometry

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ABSTRACT

This paper demonstrates that the active ingredient in many common tablets (anti-depressives, pain relievers, epileptic medicine and anti-histamines) can be recognized directly and without any pretreatment using hot cell membrane inlet mass spectrometry (hot cell MIMS). The tablets were simply placed in a sample vial and then dumped into a small oven heated to approximately 200 °C (the hot cell). The hot cell was interfaced directly to the ion source of an electron ionization mass spectrometer (EI-MS) via a polymer membrane. A few minutes thereafter an EI-MS spectrum of the chemicals desorped from the sample was recorded. Even though the tablets contained a significant number of chemicals (fillers) and often a polymer coating in addition to the active ingredients simple EI-MS spectra were recorded, wherefrom the active ingredient could be recognized by comparison with a standard EI-MS database. Of the eight tablets tested only one tablet, Bio-Melatonin (sleeping disorder), did not give a recognizable EI-MS spectrum. Over a period of 6 months the spectra recorded from the tablets did not change and the active ingredients were always recognized. The reproducibility of signal intensities from characteristic ions following successive injections was high (≈7%) for tablets without surface coating, whereas it varied up to 25% for tablets with a surface coating. Since hot cell MIMS is fully compatible with field portable instruments our results open the possibility of creating a small portable mass spectrometer for use in paramedical vehicles for immediate, on-site identification of medications found near intoxicated patients.

1. Introduction

Recently, we introduced the hot cell MIMS concept [1,2], where a solid sample can be analyzed for its potential liberation of hydrophobic organic compounds directly and without any pretreatment. The solid sample is simply placed in a sample vial, which is then immersed into a preheated hot cell (150–250 °C) interfaced directly to the ion source of an electron ionization mass spectrometer via a silicone membrane. Volatile organic compounds (VOCs) and semi-VOCs attached to the sample surface or embedded in the sample evaporate from it and diffuse through the membrane into the mass spectrometer for analysis by electron ionization mass spectrometry (EI-MS). In this fashion we have demonstrated direct analysis of pesticides attached to soil [1], analysis of phthalates and antioxidants embedded in plastics [1] and analysis of biologically active compounds (cocaine and caffeine) embedded in plant leaves [2].

The hot cell technique is in many ways complementary to the recently developed ambient ionization desorption mass spectrometry methods [3], where untreated solid samples are both desorbed and ionized in the open atmosphere. A review of the many new desorption ionization techniques was recently published [4]. The ambient ionization techniques are applicable to practically any organic chemical, although most of them work best for analysis of nonvolatile and hydrophilic compounds. Hot cell MIMS is limited to the analysis of hydrophobic VOCs and semi-VOCs, but works best for analysis of semi-VOCs in the mass range 200–500 Da. For the analysis of semi-VOCs the hot cell MIMS technique has several advantages as compared to the ambient ionization techniques. It is a very simple method that can be interfaced to most field portable instruments [5] and tentative identification of compounds can be done using standard EI-MS databases.

The compatibility of hot cell MIMS with field portable instruments and the potential identification of chemicals using standard EI-MS databases initiated this research into the possibility of creating a small portable mass spectrometer for use in paramedical vehicles for immediate, on-site identification of active ingredients in tablets found near intoxicated patients. Direct analysis of tablets is a challenging task. In some cases, like an Aspirin, the active ingredient (acetyl salicylic acid) is simply mixed and embedded in a matrix of corn starch and cellulose. In this case the active ingredient is present at the tablet surface and should be relatively easy

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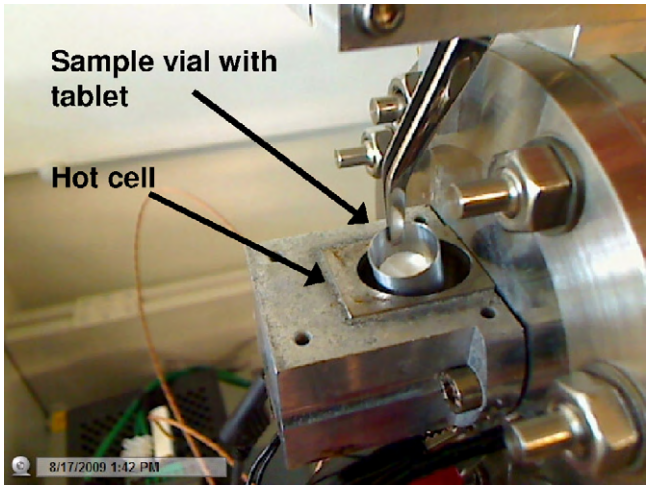

Fig. 1. Insertion of a complete tablet into the hot cell MIMS system showing the sample vial and the hot cell itself.

to release into the hot cell headspace for analysis. In other cases, like the anti-depressive Citalopram, the active ingredient is imbedded in a very complex matrix of polymers that ensures a slow release of citalopram and in addition the tablet has a surface coating that makes it easier to swallow the tablet and ensures that citalopram is not released from the tablet too early. In such cases the release of the active ingredient into the headspace of the hot cell for analysis is not straight forward. Direct analyses of active ingredients in tablets have also been performed using ambient mass spectrometry [6] and in a single case it has even been performed using a miniaturized field portable mass spectrometer [7]. In the latter case a clean spectrum with distinct protonated molecular ions from the three active ingredients (acetaminophen, acetyl salicylic acid and caffeine) was recorded using desorption electrospray ionization.

This paper is a demonstration of concept, where a number of tablets containing common pain killers, anti-depressives, anti-histamines, sleeping disorder and epileptic medication were analyzed, and the recorded hot cell MIMS spectra evaluated for their use for fast recognition of the active ingredient.

2. Experimental

2.1. Instrumentation

All experiments were conducted with a fully integrated hot cell membrane inlet/ion source system attached to a QMG 422 single quadrupole mass spectrometer (Baltzers, Liechtenstein) pumped by a single TSU 070 Turbomolecular pump (Pfeiffer Vacuum, Asslar, Germany). The details of the inlet/ion source system have been described elsewhere [1,2,5] and Fig. 1 shows a photo of the hot cell itself demonstrating the insertion of a sample vial with a tablet in. The important part of the inlet/ion source system is a 3 mm hole in the vacuum flange between the hot cell and the back side of the closed electron ionization source. The hole is covered by a flat unsupported 125 μm thick silicone membrane of medical grade (Sil-Tec Sheeting, Technical Products Inc., Decatur, GA, USA). The integrated design of the membrane inlet and the electron ion source makes it possible for VOCs and semi-VOCs to pass directly from the hot cell and into the ionizing region of the ion source without interactions with surfaces in vacuum [8,9]. The hot cell has a volume of 6 mL and is equipped with an electrical heating system that makes it possible to thermostatically control the temperature of the hot cell up to 225 °C.

2.2. Analytical procedure

The analytical procedure for analysis of the tablets was very simple. The tablet was placed in a sample vial made of alumina and then transferred into the preheated hot cell. A hot lid closed the hot cell and a mass spectrum of the VOCs and semi-VOCs liberated form the tablet into the head space was recorded 2–3 min thereafter. Following mass spectrometric analysis the sample vial was removed from the hot cell and discharged, the lid returned to the cell before laboratory air was sucked through the hot cell via 2 holes in the lid using a standard laboratory air suction pump (1 L/min). The use of a sam-

Table 1
An overview of tablets tested with information of pharmaceutical effect, active ingredient and tablet material.

Tablet	Pharmaceutic effect	Active ingredient	Total amount (mg)[a]	Tablet materials[b]
Aspirin	Pain reliever	Acetyl salicylic acid	500	Corn starch, cellulose
Ibumetin	Pain reliever	Ibuprofen	400	Hypromellose, potato starch, sodium cross caramellose, lactose monohydrate, magnesium stearate, micro crystalline cellulose, propylene glycol, colloid silica
Pinex	Pain reliever	Paracetamol	500	Hydroxypropylcellulose, corn starch, magnesium stearate
Citalopram	Antidepressive	Citalopram hydrobromide	40	Cellulose, glycerol, corn starch, lactose monohydrate, magnesium stearate, copolyvidone, sodium cross caramellose, macrogol 6000, hypromellose, titanium dioxide
Venlix	Antidepressive	Venlafaxine hydrochloride	37.5	Micro crystalline cellulose, lactose monohydrate, sodium starch glycolate, magnesium stearate, yellow and brown ironoxide
Prometazin-ERA	Antihistamine	Prometazine hydrochloride	25	Micro crystalline cellulose, potato starch, lactose monohydrate, povidon, ethanol (96%), water, magnesium stearate
Phenemal-DLF	Anti-epileptic	Phenobarbital	100	Gelatin, potato starch, magnesium stearate, lactose monohydrate, talcum
Bio-Melatonin	Sleeping disturbance	Melatonin	3	Cellulose, Silica, Stearic Acid, Cellulose Gum, Magnesium Stearate

[a] Total amount of active ingredient in the tablet.
[b] Tablet materials (coatings inclusive) as stated in the contents declaration of the packet.

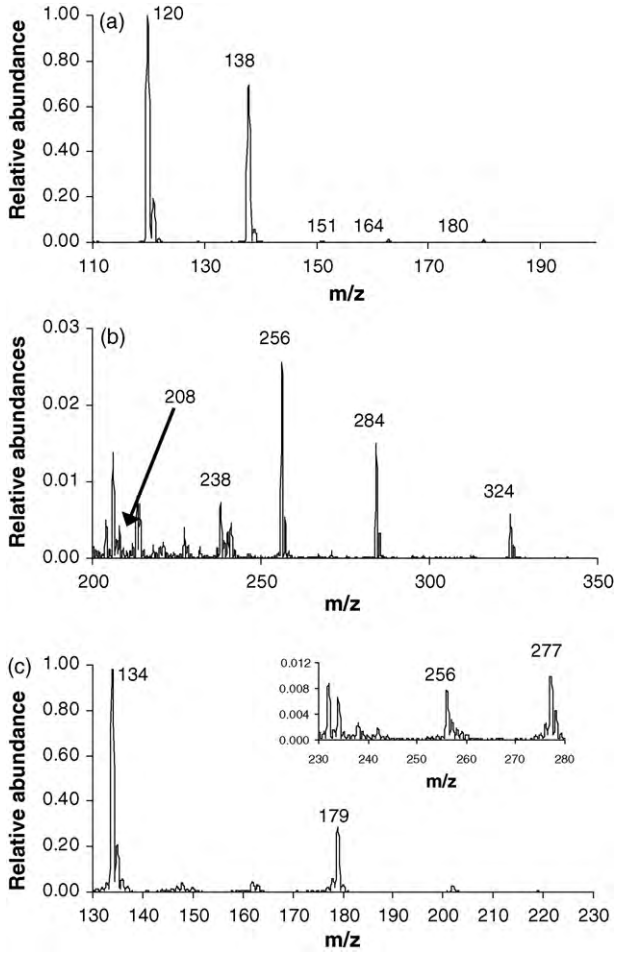

Fig. 2. EI-MS spectra recorded directly from (a) Aspirin (acetyl salicylic acid), (b) Citalopram (citalopram hydrobromide) and (c) Venlix (venlafaxine hydrochloride) tablets. The relative abundances in the three spectra are directly comparable.

ple vial to hold the tablets reduced the carryover from one sample to the next considerably, since no tablet material is left in the hot cell between two successive analyses. This is particular important in connection with tablet analysis, since some of the tablets melted upon heating to 200 °C. Memory effects caused by binding of sample molecules to the membrane and other exposed surfaces are reduced by the flushing of the cell with laboratory air. In this fashion the complete analytical procedure, analysis and preparation of the hot cell for the next sample took less than 10 min.

2.3. Chemicals

The following tablets available on stock at the Faculty of Pharmaceutical Sciences, University of Copenhagen were used in this study: Aspirin, Ibumetin, Pinex, Citalopram, Venlix, Prometazin-Era, Phenemal-DLF and Bio-Melatonin. A detailed description of the tablets is given in Table 1.

3. Results and discussion

Fig. 2a shows the EI-MS spectrum recorded from an Aspirin, a typical non-prescriptive pain reliever. The spectrum is an almost perfect match to the NIST database [10] except for the barely visible peak at m/z 151, which originates from the tablet material. In cases like this, where the amount of the active ingredient is in the range of 500 mg and the tablet only contains a few fillers like corn starch and cellulose, recognition of the active ingredient in the tablet is straight forward using database search. It is more difficult, when the amount of the active ingredient becomes lower and the compound becomes larger and less volatile. This is demonstrated in Fig. 2b that shows the EI-MS spectrum obtained from a tablet containing 40 mg of the anti-depressive citalopram. The presence of citalopram is recognized by the molecular ion at m/z 324 and two characteristic high mass fragments at m/z 208 and m/z 238. In this case the EI-MS spectrum shows a small deviation from the NIST database. The molecular ion is approximately 2.5 times higher in intensity relative to the ions from the fragments at m/z 208 and m/z 238 as expected from the NIST database. This difference is the result of the slow scan rate (5 Da/s) of our mass spectrometer combined with increased liberation of active ingredient from the tablet as it heats up. At the time where the molecular ion is detected, the concentration of citalopram inside the hot cell is higher than it was at the time where the two fragments were detected. Detection of venlafaxine from Venlix tablets (Fig. 2c) represents another challenge to hot cell MIMS with electron ionization. In electron ionization venlafaxine fragments so much that the abundance of the molecular ion is too low for registration in the NIST Database. Despite this, we still observe a distinct molecular ion (see insert in Fig. 2c).

The complex composition of the citalopram tablet material (see Table 1) causes a considerable background of ions all the way up to m/z 300, see for example the ions at m/z 256 and m/z 284 in the recorded citalopram spectrum and again m/z 256 in the recorded spectrum of venlafaxine. All the constituents in the tablet material are hydrophilic and have a relatively high molecular weight, so we do not expect them to be able to penetrate the highly hydrophobic silicone membrane. Instead we believe that the background ions are the result of chemical reactions between the tablet constituents at the high temperature inside the hot cell. The presence of these background ions complicate the recognition of active ingredients in a tablet of unknown origin, and we suggest a strategy, where a database with standard hot cell MIMS EI-MS spectra of a few hundred common tablets are used to search the spectrum for distinct molecular ions and at least two other high molecular weight fragments. This "safe" strategy of requiring a clear presence of the molecular ion has a significant price in the form of reduced sensitivity for compounds like citalopram and venlafaxine, where the molecular ion has an abundance more than 50 times lower than the most abundant fragment ion.

To test the general applicability of the method for fast recognition of tablets in the field, we analyzed a number of different tablets covering common non-prescriptive pain relievers, anti-depressives, anti-histamines, anti-epileptic and sleeping disorder medications. In all cases the tablet was inserted into the hot cell at 200 °C and the EI-MS spectrum was recorded 3–4 min thereafter. The chosen hot cell temperature of 200 °C was a compromise. In general [1,2] the higher the temperature of the hot cell the more volatile the analytes become with increased signal as the result, but at the same time the number of background ions from the tablet material increase, especially at lower masses. Small and more volatile analytes like acetyl salicylic acid are therefore normally best analyzed at hot cell temperatures around 170 °C, whereas large and less volatile compounds like citalopram are normally best analyzed at temperatures around 220 °C [1,2,5]. Under all circumstances an elevation of the hot cell temperature increases the risk of a thermal degradation of the active ingredient.

Table 2 shows the result of the tablet screening. With the exception of melatonin, the rest of the active tablet ingredients were easily recognized from their tablets using the criterion of a positive identification of the molecular ion plus at least two characteristic "high mass" fragment ions. Even acetyl salicylic acid, citalopram, venlafaxine and promethazine that fragment considerably, with expected abundance of molecular ions less than 2% of the main EI ions gave recognizable ion patterns. Melatonin is structurally simi-

Table 2
A list over measured active ingredients in the tablets with indication of the ions used for their recognition.

Active ingredient (CAS. no.)	Molecular weight	Formula	EI ions used for identification[a] *m/z* (%)
Acetyl salicylic acid (50-78-2)	180	$C_9H_8O_4$	Measured ions: 120(100), 138(67), 180(1) NIST database: 120(100), 138(58), 180(2)
Paracetamol (103-90-2)	151	$C_8H_9NO_2$	Measured ions: 109(100), 151(40), 152(4) NIST database: 109(100), 151(44), 152(4)
Ibuprofen (15687-27-1)	206	$C_{13}H_{18}O_2$	Measured ions: 161(100), 163(85), 206(57) NIST database: 161(100), 163(88), 206(39)
Citalopram (59729-33-8)	324	$C_{20}H_{21}FN_2O$	Measured ions: 208(57), 238(100), 324(75) NIST database: 208(90), 238(100), 324(31)
Venlafaxine (93413-44-6)	277	$C_{17}H_{27}NO_2$	Measured ions: 135(100), 179(24), 277(0.5) NIST database: 135(100), 179(17), 277(<0.5)
Promethazine (60-87-7)	284	$C_{17}H_{20}N_2S$	Measured ions: 180(94), 214(100), 284(55) NIST database: 180(100), 214(67), 284(75)
Phenobarbital (50-06-6)	232	$C_{12}H_{12}N_2O_3$	Measured ions: (100), 205(20), 232(24) NIST database: 204(100), 205(12), 232(12)
Melatonin (73-31-4)	232	$C_{13}H_{16}N_2O_2$	Measured ions: not detected NIST database: 160(100), 173(93), 232(17)

[a] Appearance of the molecular ion and two high mass fragments in the EI-MS spectra recorded from the tablets and for comparison the same ions as given in the NIST Chemistry Webbook [10]. The relative abundances of the ions are relative to the most abundant ion of the three ions chosen for recognition of the active ingredient.

lar to some of the other compounds tested and should be detectable with the hot cell MIMS. We expect that the missing recognition of melatonin is the result of a much lower content of active ingredient (3 mg) than that in the other tablets. Based upon this observation and an evaluation of the signal to noise ratio in the mass spectra of the other compounds (see for example citalopram Fig. 2b) we estimate a general detection limit to be around 5 mg total content of active ingredient in the tablet.

The direct analysis of chemicals liberated from solid materials using hot cell MIMS is a fast screening method. It is not very quantitative, since the signals in addition to analyte concentration also depend upon sample morphology, sample matrix, amount of sample and whether the analyte is present on the surface of the sample or imbedded inside the sample. However, if the method is to be used for fast on-site recognition of the active ingredients in unknown tablets, then the method must be reproducible enough for positive recognition of the active ingredients every time. To test this all the tablets have been analyzed numerous times over a period of 6 months and always with a positive result (except for melatonin). During the 6 months period very little service to the instrument was needed, only a few times where tablet material slipped out of the sample vial and into the hot cell itself, cleaning of the hot cell and replacement of the membrane was necessary.

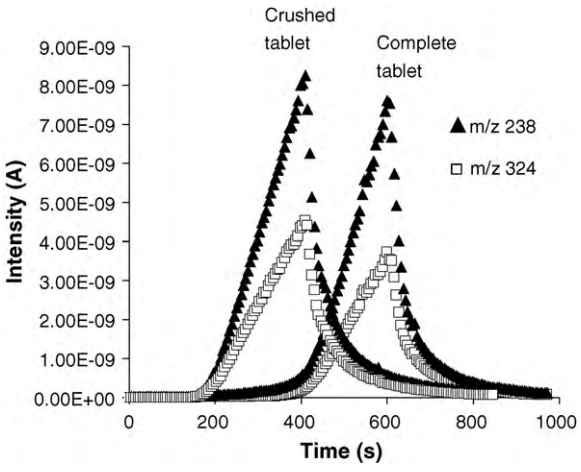

Fig. 3. Multiple ion monitoring detection of characteristic ions from citalopram following the insertion of a crushed and a complete Citalopram tablet.

Some of the tablets, Ibumetin, Pinex, Prometazin-ERA, Citalopram, BIO-Melatonin and Phenemal-DLF, have a surface coating that makes it easier to swallow the tablet. To test the influence of this surface coating upon the recorded EI-MS spectra, we performed a multiple ion monitoring experiment of *m/z* 324 and *m/z* 238 from citalopram following the insertion of an intact tablet or a crushed tablet into the hot cell (see Fig. 3). Basically, the signal response from a complete tablet and a crushed tablet is identical apart from a 2 to 3 min delay in the appearance of the signal from the intact tablet. It is clear that the surface coating needs to break before we can record a signal from the active ingredient and we expect that expansion of the filling material during the heating process caused the tablet coating to burst. As a result of the need for a rupture of the tablet coating the reproducibility of signal intensities from surface coated tablets was relatively poor (about 25%) as compared to a high reproducibility for uncoated tablets (about 7%).

4. Conclusion

This paper demonstrates that hot cell MIMS can be used for fast and direct recognition of the active ingredient in tablets commonly taken as an overdose. Both tablets with and without a surface coating can be analyzed within minutes and without any pretreatment, but in the case of coated tablets the appearance of the signal is typically delayed by 2–3 min unless the tablet material is broken or crushed. Over a period of 6 months very little service to the instrument was needed and the active ingredients in the tablets analyzed were always recognized. As a next step in our research, we intend to implement the hot cell MIMS technique into a smaller field portable quadrupole mass spectrometer for actual field testing under harsh conditions.

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