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Determination of zearalenone and its metabolites α- and β-zearalenol in beer samples by high-performance liquid chromatography–tandem mass spectrometry

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Abstract

A fast, robust and sensitive LC–MS–MS method for the determination of zearalenone (ZON) and its metabolites α-zearalenol (α-ZOL) and β-zearalenol (β-ZOL) in beer samples is described. Sample preparation was performed by direct RP-18 solid-phase extraction of undiluted beer samples followed by selective determination of analytes by LC–MS–MS applying an atmospheric pressure chemical ionization (APCI) interface. Using the negative ion mode limits of determination of 0.03–0.06 μg l^{-1} beer and limits of quantification of 0.07–0.15 μg l^{-1} beer were achieved, which was distinctly more sensitive than in the positive ion mode. Twenty-three beer samples from different countries, produced from different grains and under different brewing conditions, were investigated by this method, but only in one sample could β-ZOL and ZON be detected. Independently of the type of beer, relative standard deviations between 2.1% and 3.3%, a linear working range of 0.15 μg l^{-1} to 500 μg l^{-1} beer and recovery rates around 100% could be achieved when zearalanone (ZAN) was used as internal standard.

Keywords: Zearalenone; Zearalenol

1. Introduction

Mycotoxins are secondary metabolites produced by fungal species, growing on agricultural products during cultivation, harvest, transport and storage [1,2]. Their occurrence in food has been recognized as potential human health hazard either caused by direct contamination of grains and fruits and their products or by ''carry over'' of mycotoxins and their metabolites in animal tissues [3–6]. Some hundred mycotoxins have been identified so far, mostly produced by *Fusarium*, *Aspergillus* and *Penicillium* fungal species. Due to their frequent occurrence and their severe toxic, estrogenic and cancerogenic properties, guidelines and tolerance levels of these compounds have been set for feeding stuff and foods in several countries [7].

Zearalenone (ZON, Fig. 1) is produced by *Fusarium* species, which colonize several grains [8]. High amounts of ZON can most frequently be found on maize, wheat, oats and barley. It has relatively low acute toxicity [9] and its carcinogenic properties are controversially estimated [10,11]. On the other

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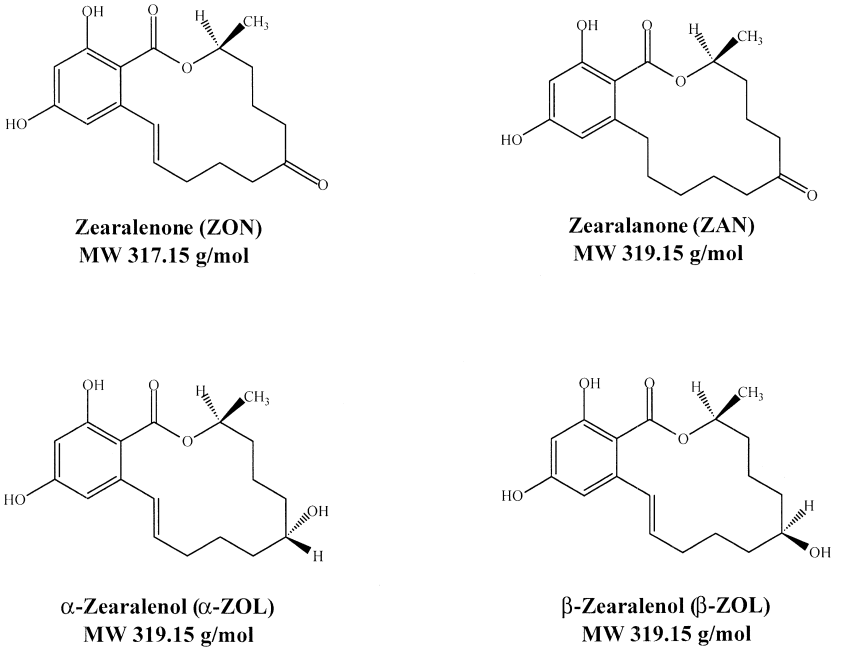

Fig. 1. Chemical structures of *Fusarium* mycotoxins investigated.

hand, it exhibits distinct estrogenic and anabolic properties in several animal species. This is due to its ability to couple with the estrogenic receptor, resulting in severe effects on the reproductive system [2,12]. Tolerance levels in grains and grain products have been set in several countries with a concentration range from 30 to 1000 μg kg^{-1} [7]. ZON is partially metabolized (reduced) to structurally and stereochemically related α- and β-zearalenol (α- and β-ZOL, Fig. 1) in the liver of animals or humans. While the estrogenic properties of β-ZOL are comparable to ZON, the estrogenic effect of α-ZOL is approximately three-times stronger [13].

ZON may be transmitted from contaminated grains into beer during the brewing process. A study on Nigerian traditional brewing of corn beer showed a 51% carry over of ZON from malt homogenate to finished product [14]. Resulting from that, ZON was found at high incidence and concentrations in African beers [15,16], which is in distinct contrast to surveys of European [17,18], Canadian [19] and Korean beers [20], when only one beer sample was determined to contain ZON at a level of 100 μg l^{-1} [18]. Besides, it was shown that a part of ZON was converted to α- (8%) and β-ZOL (69%) by brewing strains of *Saccharomyces cerevisiae* during the brewing process [21,22]. However, α- and β-ZOL have not been found in any other investigation of beer samples.

So far, thin-layer chromatography (TLC) [23], high-performance liquid chromatography (HPLC) [24–26], gas chromatography (GC) [13] and enzyme-linked immunosorbent assay (ELISA) [27] have been applied for the determination of ZON and of ZOLs. Compared to other analytical methods, LC-based methods offer often the advantage of a reduction of time-consuming and sometimes error prone sample preparation and derivatisation steps. In addition, chromatographic methods also allow the simultaneous investigation of several mycotoxins in a single run [13,24,25]. In this context, HPLC has been used for ZON and of ZOL analysis enabling a detection limit in the low μg kg^{-1} range with fluorescence detection [26].

Extensive and/or very selective sample clean-up procedures are necessary to remove on the one hand main parts of matrix compounds that may interfere with the analyte signal when using UV and fluorescence detection and on the other hand to preconcentrate the analytes in order to reach the required low

determination limits. Classical methods for the clean-up of ZON and of α-/β-ZOL containing samples, such as liquid–liquid partitioning [26], are considered time-consuming, frequently inaccurate and consume relatively large volumes of chlorinated solvents. Recently, solid-phase extraction (SPE) with immunoaffinity material has become popular in mycotoxin analysis as a very selective and time-saving one-step sample clean-up tool [26,28] enabling almost complete removal of possibly interfering matrix compounds. However, multi-toxin analysis is not feasible with these columns, since they are highly specific for only one target mycotoxin (family). In addition, immunoaffinity materials are considered expensive compared to conventional and less selective SPE materials.

An alternative approach to the reduction of sample preparation and, simultaneously to enhance sensitivity of an analytical method, is to use a highly selective and sensitive detector system. Mass spectrometry (MS) meets these demands very well and offers the additional advantages of its universal applicability to a wide variety of different analytes and its suitability for multi-analyte detection. Laborious and time-consuming sample clean-up procedures may be minimized to a great extent or even completely omitted, since co-eluting matrix compounds may be eliminated by MS selectivity. Single-ion monitoring (SIM) and multi-reaction monitoring (MRM) with tandem MS instruments (MS–MS) enable a specific and exact determination with a wide linear range.

In mycotoxin analysis, particularly dealing with ZON and α-/β-ZOL determination in different matrices, MS has only frequently been used in combination with GC (GC–MS and GC–MS–MS) [29,30] achieving detection limits of 2 μg l^{-1} for ZON and ZOLs in beer [19,20]. However, also on-line coupling of mass spectrometry with HPLC (LC–MS) has been described using a robust atmospheric pressure chemical ionization (APCI) interface [31]. Detection limits of ZON in the low μg l^{-1} range were achieved in the SIM mode [32] and the ng l^{-1} range in the MRM mode [33]. SPE with either immunoaffinity material or RP-18 material was used for an effective one-step sample clean up of maize samples giving comparable good results when a highly selective MS–MS detection system was applied. This has been demonstrated for ZON analysis, however, not for simultaneous ZON/ZOL analysis.

In this paper the simultaneous determination of ZON, α- and β-ZOL in beer in the μg l^{-1} to ng l^{-1} range is described. This is achieved with a simple one-step sample clean-up by RP-18 SPE followed by a LC–MS–MS analysis in the negative ion mode. In order to evaluate whether different beer matrices have impact on the ionization capability of the APCI interface, 23 different beer samples were investigated. Particular attention was given to the validation of the multi-analyte method in food samples. This includes aspects related to major implications of a relatively unselective sample clean-up on reliable LC–MS–MS detection and quantitation. Accuracy of data obtained with external and internal standard method were compared.

2. Experimental

2.1. Materials

ZON, α-ZOL, β-ZOL and zearalanone (ZAN, internal standard, I.S.) were purchased from Sigma (Deisenhofen, Germany). Ammonium acetate (analytical-reagent grade) as well as HPLC grade methanol and HPLC grade acetonitrile were supplied by Merck (Darmstadt, Germany). Samples of bottled beer produced in Australia, Austria, Czech Republic, England, Germany, Mexico, The Netherlands, Nigeria, Scotland, Zimbabwe, South Korea, People's Republic of China, USA and Vietnam were obtained from local stores (Vienna, Austria). SPE columns filled with 100 mg of reversed-phase material (RP-18, 20–40 μm) were purchased from Phenomenex (Torrance, CA, USA). Water (conductivity of 18 $M\Omega^{-1}$ cm^{-1}) was purified by an Elgastat water purification system (Bucks, UK). HPLC solvents were filtered before use through GF/A glass microfiber filters (Whatman, Maidstone, UK).

2.2. Instrumental

LC–MS–MS analyses were performed on a PE Sciex API 365 LC–MS–MS system (Perkin Elmer Sciex Instruments, Thornhill, Canada) equipped with a Hewlett-Packard HPLC system, Model 1100 series

(Hewlett-Packard, Waldbronn, Germany) and with an APCI interface. Chromatographic separation was achieved on a 15 cm×3 mm I.D. HP/HPV Shield RP-8 column (Waters, Milford, MA, USA), protected by a LiChroCART guard column (Merck) packed with LiChrospher 60 5 μm RP-select B material (Merck). A mobile phase of 15 m*M* ammonium acetate in methanol–water (65:35, v/v) was used at a flow-rate of 0.5 ml min^{-1}. The retention times for α-ZOL, β-ZOL, ZON and ZAN were 11.38, 6.87, 10.08 and 9.02 min, respectively (capacity factors, 7.59, 4.58, 6.72 and 6.01). The APCI interface was applied in the negative ion mode at a temperature of 400°C and with a needle current of 4 μA. For MRM the deprotonated molecular species of ZON (*m*/*z* 317.15) and of α-ZOL, β-ZOL, ZAN (*m*/*z* 319.15) were used as precursor ions and the following fragment ions were selected: *m*/*z* 131.1, 175.1 for ZON, *m*/*z* 275.1 for α-/β-ZOL and *m*/*z* 205.1 for ZAN. The dwell time was 0.8 s for each fragmentation pathway. The collisional energy was adjusted by variation of the voltage difference between the high-pressure entrance quadrupole (Q0) and the collisional cell quadrupole (RO2) and was found to give an average highest sensitivity for all three analytes with a value of 30 eV. Nitrogen was used as collisional gas.

2.3. Sample preparation

Stock solutions of ZON, α-ZOL, β-ZOL and ZAN ranging from 10 to 20 mg l^{-1} in methanol were prepared from pure standards. These solutions were used to make mixed standard solutions of all three analytes and of the internal standard ranging from 52.5 to 525 μg l^{-1}, which were stored at 4°C under exclusion of light. For calibration and spiking experiments these standard solutions of the internal standard and the three analytes were added to the beer samples directly before sample preparation was carried out.

A 50-ml beer sample spiked with internal standard and analytes was degassed with a stream of dry nitrogen for 30 min and filtered through a GF/A glass microfibre filter (Whatman), A 9-ml sample aliquot was applied to a RP-18 SPE column which had first been activated with 5 ml of methanol followed by 10 ml of water. After sample loading, the SPE column was washed with 2 ml of water. For complete elution of analytes and internal standard, 1.25 ml of 10 m*M* of ammonium acetate in methanol–water (70:30, v/v) was used. This fraction was evaporated under a stream of nitrogen and the residue redissolved in 250 μl 15 m*M* ammonium acetate in methanol–water (65:35, v/v). After filtration through a 0.45 μm Millex-HV filter (Millipore, Eschborn, Germany), 50 μl of this solution were injected into the HPLC–MS–MS system.

3. Results and discussion

Based on earlier investigations with ZON [32,33], the negative ion mode was used for the determination of α- and β-ZOL and was found to be by a factor of 10 more sensitive than the positive ion mode. To use the negative ion mode seems to be generally advantageous for compounds containing phenolic groups as, e.g., stilbenes also were found to give strikingly more abundant negatively charged deprotonated molecular ions than positively charged protonated molecular ions [34]. On the other hand, ion abundances are distinctly more scattered (10%) than in the positive ion mode. Therefore, ZAN was used as internal standard to avoid excessive external calibration procedures. It exhibits similar physical and chemical properties as ZON and the ZOLs and was well suited to reduce scattering of data resulting either from the sample clean-up, from matrix effects or from the ionization process.

One of the main problems of MS detection dealing with similar compounds is the formation of identical molecular ions in SIM mode or identical ion pairs in MRM mode by two or several molecular species. As ZAN and α- and β-ZOL contribute to the identical *m*/*z* 319.15⇒275.1 fragmentation pathway, an improved chromatographic separation prior to mass spectrometric analysis was necessary for such a type of multi-analyte determination. Sufficient resolution was achieved using a ''shielded'' RP-8 column. The appearance of ZON does not interfere with the ZOLs and ZAN (see also Fig. 2B). Other RP-8 and RP-18 phases were less selective for this application.

Sample clean-up was performed in one step by applying directly degassed beer samples to RP-18 SPE columns achieving sufficient enrichment of all

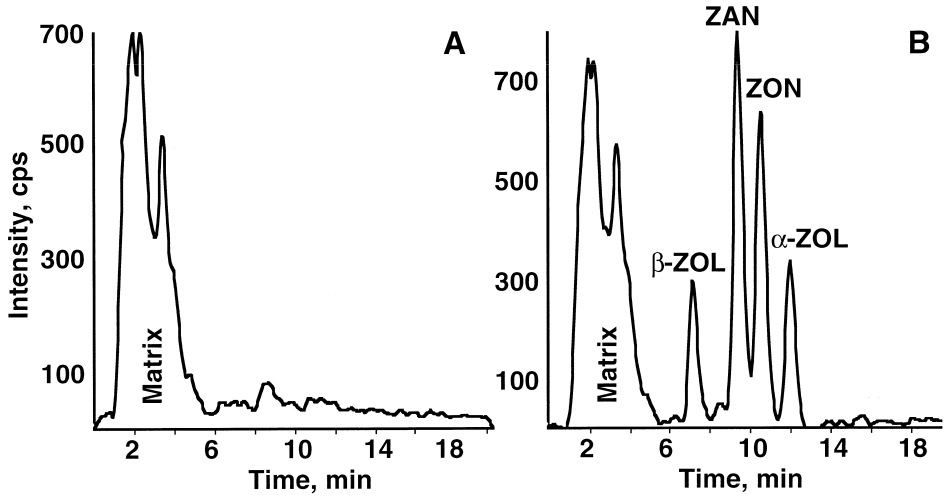

Fig. 2. (A) MRM chromatogram of an unspiked beer sample (Edelweiss, Austria). (B) MRM chromatogram of the same beer sample spiked with ZON, α-ZOL, β-ZOL (each 3.1 μg l^{-1}) and with ZAN (31 μg l^{-1}). MRM ions for ZON (317.1⇒131.1; 317.1⇒175.1), for α-/β-ZOL (319.1⇒275.1) and for ZAN (319.1⇒205.1).

analytes and the internal standard (ZON, α-ZOL, β-ZOL, ZAN). Overall recovery rates for ZON, α-ZOL, β-ZOL and ZAN of 104%, 106%, 99% and 98%, respectively, were obtained. Method limits of determination (LODs) were 0.03 μg l^{-1} beer for ZON and 0.06 μg l^{-1} beer for both ZOLs. Method limits of quantification (LOQs) of 0.06 μg l^{-1} beer for ZON and 0.15 μg l^{-1} beer for both ZOLs could be achieved with a linear range between LOQs and up to 500 μg l^{-1} beer.

As previously shown for the determination of ZON in maize samples, SPE of less selective RP-18 material in comparison to highly ZON selective immunoaffinity material exhibit the same performance in terms of accuracy, sensitivity and reproducibility when used in combination with the highly selective LC–MS–MS detection system [33]. Besides, RP-18 materials offer the major advantage to be much better suitable for multi-analyte determination. This could be clearly demonstrated for the determination of ZON and its metabolites α-/β-ZOL in beer samples.

In Fig. 2A a typical total-ion chromatogram of a non-contaminated beer sample is shown. The non-selectivity of the one-step sample preparation is reflected by several high abundant matrix peaks at the front of the chromatogram. However, the separation of all four analytes from the matrix prevent interferences of any kind as depicted in Fig. 2B. This finding indicates again that chromatographic separation prior to mass spectrometric analysis is mandatory for the given task, even for the relative selective MRM detection mode.

In routine use, the relative crude sample purification resulted in a slow blockage of the APCI interface due to carbon precipitation. This relates to significant amounts of matrix components which are pyrolized within the quartz tube of the interface during the evaporation process. From a practical point, the APCI interface had to be cleaned every 100 samples, which accounted for about two extra hours of system maintenance work.

A further problem of MS detection and quantification may be matrix effects on the MS detector response which is rarely discussed in literature. The common perception is that LC–MS–MS guarantees high selectivity paired with low incidence of interferences. Consequently, sample preparation may be strongly reduced or even eliminated, and only a little chromatographic separation is required to isolate the analytes from each other or from the matrix. Contrary to this common belief, some authors have recently reported that co-eluting matrix compounds can severely affect the ion formation process in ESI and APCI interfaces resulting in a decrease of accuracy and reproducibility of LC–MS–MS analy-

ses [35]. To avoid such related ion suppression phenomena it was suggested to incorporate more efficient sample clean-up and/or to improve chromatographic separation to remove co-eluting compounds that may disturb the LC–MS–MS analysis [36,37]. Alternatively, suitable internal standards could be applied to eliminate efficiently matrix effects. Co-eluting isotope labeled (deuterated) standards of the analytes should be best suited, but they are either not commercially available or very expensive. As we have found earlier [33] ZAN fulfills reliably the requirements for an internal standard, due to its similar chemical and physical properties compared to ZON and the ZOLs. For grain samples the relative standard deviation (RSD) improved significantly compared to the external standardization. The present study confirms these results.

The present LC–MS–MS method was applied to the analysis of ZON, α-ZOL and β-ZOL in 23 different beer brands from European, Asian, African and American countries, as listed in Table 1. It reflects different grains and other raw materials and brewing procedures. Calibration functions were established for each sample matrix to estimate the robustness of the total method.

3.1. Method validation

Method validation was based on spiking each beer brand with the analytes and the internal standard within the calibration range and judging the recovery values with regard to the expected analysis values. These results are listed in Table 2. Accuracy of data obtained for external calibration is poor and deviations of obtained values from mean values ranged from −15 to +40%. This finding may be related to the above mentioned variations of about 10% of the detector response. On the other hand, they clearly reflect also matrix effects since repeated analyses of the same beer sample resulted in similar deviated data. Applying the internal standard method, both the variation of the detector response as well as the matrix effects, were greatly eliminated for all investigated matrices and for all analytes (0.2–5.0% deviations of obtained values from mean values). Such a methodological improvement is not necessarily al-

Table 1
Details of beer brands investigated for mycotoxin contamination

Beer sample	Country	Raw materials	Alcohol content (%)	Brewing process
Mönchsgold	Austria	Malt, hops	5.0	Lager
Ottakringer	Austria	Malt, hops	4.2	Ale
Eggenberger	Austria	Malt, hops	9.6	Ale
Pilsner	Austria	Malt, hops	5.0	Lager
Edelweiss	Austria	Wheat, yeast, hops	5.5	–
Clausthaler	Germany	Malt, yeast, hops	<0.5	Lager
Erdinger	Germany	Wheat, yeast, hops	5.3	–
Vitamalz	Germany	Malt from barley, syrup, hops	0.0	–
Amstel	The Netherlands	Malt, hops	No details given	Lager
Adelscott	England	Wiskey malt, hops	8.5	–
Newcastle	England	Wheat, syrup, sugar	4.7	Brown ale
Miller	USA	Malt, maize, hops	4.7	Lager
Foster	Great Britain	No details given	5.0	Lager
Tennent	Scotland	No details given	5.0	–
Guinness	Ireland	Malt, yeast, hops	5.0	Stout
Spendrup	Sweden	Malt, yeast, hops	5.0	Lager
Sapporo	Japan	Malt, maize, hops	4.5	Lager
Hite	South Korea	Malt, yeast, hops	4.5	–
Hrlidr	Vietnam	No details given	5.0	–
Tsingtao	China	Malt, rice, hops	4.5	–
Corona	Mexico	No details given	4.6	–
Zambezi	Zimbabwe	Malt, maize, hops	4.5	–
Nigeria Pal	Nigeria	Malt, maize, hops	5.0	–

Table 2
Results of the determination of ZON, α- and β-ZOL in 23 different beer samples comparing external and internal standard (I.S.) methods[a]

Beer sample (n=23)	Spiked values ($\mu g\ l^{-1}$)			Obtained values with I.S. ($\mu g\ l^{-1}$)			Deviation of obtained from spiked values with I.S. (%)			Deviation of obtained from spiked values without I.S. (%)		
	α-ZOL	β-ZOL	ZON	α-ZOL	β-ZOL	ZON	α-ZOL	β-ZOL	ZON	α-ZOL	β-ZOL	ZON
Mönchsgold	55.6	55.6	58.4	53.9	53.7	58.5	−3.0	−3.4	+0.3	+0.5	+0.4	+5.0
Ottakringer	55.6	55.6	58.4	57.2	56.1	55.7	+3.1	+1.2	−4.2	+5.2	+3.8	−1.5
Eggenberger	55.6	55.6	58.4	53.0	53.1	58.5	−4.5	−4.4	+0.2	+3.0	−4.4	−0.7
Pilsner	55.6	55.6	58.4	52.8	53.9	57.9	−4.9	−2.9	+0.9	+1.2	+4.7	+6.9
Edelweiss	55.6	55.6	58.4	58.0	58.2	57.9	+4.2	+4.4	−1.0	−9.3	−12.3	−15.5
Clausthaler	55.6	55.6	58.4	53.6	53.7	55.7	−3.5	−3.3	−4.4	+17.2	+23.1	+21.9
Erdinger	55.6	55.6	58.4	57.1	56.5	60.4	+2.7	+1.7	+3.7	–	–	–
Vitamalz	55.6	55.6	58.4	54.4	57.7	57.3	−2.1	+3.9	−1.8	−8.4	+1.1	−6.8
Amstel	55.6	55.6	58.4	55.3	52.8	57.1	−0.4	−4.9	−2.2	−0.1	−4.9	−1.8
Adelscott	55.6	55.6	58.4	55.2	56.3	57.6	−0.7	+1.3	−1.2	–	–	–
Newcastle	55.6	55.6	58.4	54.8	52.8	58.0	−1.3	−5.0	−0.6	+3.0	−2.0	+3.3
Miller	**55.6**	**55.6**	**58.4**	**55.0**	**55.7**	**59.3**	**−0.7**	**+1.6**	**+1.2**	**−3.5**	**+0.4**	**−1.7**
Foster	55.6	55.6	58.4	55.5	55.6	56.9	−0.2	−0.1	−2.5	–	–	–
Tennent	55.6	55.6	58.4	53.4	53.2	56.9	−3.9	−4.3	−2.4	−0.3	−0.9	+0.6
Guinness	55.6	55.6	58.4	57.3	53.6	59.3	+3.1	−3.5	+1.6	+11.1	+1.8	+5.7
Spendrup	55.6	55.6	58.4	56.6	58.2	58.0	+1.8	+4.8	−0.6	+6.0	+12.9	+3.8
Sapporo	55.6	55.6	58.4	56.1	57.5	59.3	+0.9	+3.4	+1.7	+10.6	+5.6	+3.9
Hite	55.6	55.6	58.4	53.3	52.9	56.9	−4.0	−4.9	−2.5	+32.9	+30.4	+36.7
Hrlidr	55.6	55.6	58.4	58.4	56.1	59.8	−1.3	−1.0	+2.4	+1.3	−1.1	+0.3
Tsingtao	55.6	55.6	58.4	58.0	55.7	57.8	+4.4	+0.2	−1.0	+11.4	+1.3	+13.0
Corona	55.6	55.6	58.4	57.2	57.1	59.2	+3.0	+2.8	+1.6	+40.2	+40.0	+34.5
Zambesi	55.6	55.6	58.4	54.2	54.9	58.1	−2.5	−1.3	+0.5	+15.9	+17.6	+18.8
Nigeria Pal	55.6	55.6	58.4	56.8	54.9	56.9	+2.2	−1.2	−2.5	−0.2	−3.5	−4.7

[a] In Miller beer (bold) 0.264 $\mu g\ l^{-1}$ of β-ZOL and some ZON below the LOQ were found. α-ZOL RSD=3.2%; β-ZOL RSD=3.3%; ZON RSD=2.1%. Recovery: α-ZOL 106%; β-ZOL 99%; ZON 104%.

ways the case, as previously shown for the determination of finasteride in human plasma, when only removal of compounds co-eluting with the internal standard and/or the analyte by an improved chromatographic separation or more efficient sample clean-up was suitable to eliminate this problem [36]. Elimination of matrix effects was, however, only possible for the analysis within each beer brand. As exemplified for β-ZOL in Fig. 3, calibration curves of all analytes were considerably different in individual beer brands indicating that the internal standard was not able to compensate for all matrix effects between diverse beer brands. This prevents the application of a general calibration function for all beer brands. This limitation may be avoided when a more selective sample clean-up is applied to remove disturbing matrix compounds. The disturbance can be initiated either by coelutes of the analytes but not of the internal standard, or vice versa, or by unequal disturbance of analytes and internal standard due to various amounts of coelutes.

The described LC–MS–MS method was applied to a number of beer brands to investigate possible natural ZON/ZOL contamination. As a result only one sample – Miller, USA (bold in Table 2) brewed from maize – out of 23 beer samples was found to contain β-ZOL (0.264 $\mu g\ l^{-1}$ beer) and ZON (below LOQ of 0.06 $\mu g\ kg^{-1}$). In none of the samples α-ZOL could be detected.

4. Conclusion

The combination of RP-18 SPE and sample clean-up with a highly selective LC–MS–MS analysis method proved to be of broad applicability in the field of selective and sensitive ZON/ZOL multi-compound analysis. This was demonstrated by the

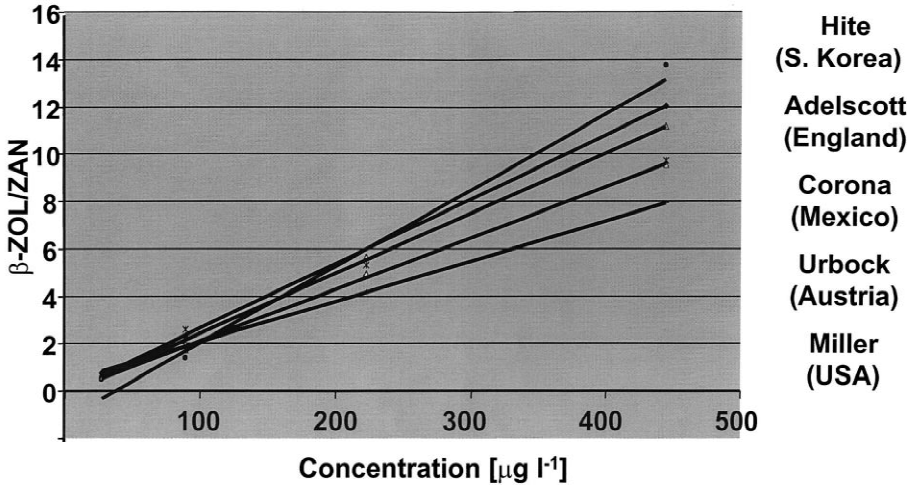

Fig. 3. β-ZOL calibration curves of five selected beer brands. The area ratio of β-ZOL and the internal standard ZAN was used for calculation of calibration curves.

investigation of 23 different beer samples. The agreement of measured and expected values were excellent proving that this method is independent of the various beer matrices. Internal calibration is, however, limited to a particular beer brand. Using a general calibration curves for all beer brands is, therefore, not possible, since calibration curves vary considerably from brand to brand. A possible solution to this problem is a more efficient removal of matrix compounds by e.g., a more selective solid-phase extraction procedure with e. g. immunoaffinity materials.

Implementing the negative ion mode seems to be of general applicability also to other mycotoxins and xenobiotics containing phenolic hydroxy groups or carboxy groups, as it was demonstrated in preliminary experiments with stilbene anabolics, but also with ochratoxins.

However, certain potential limitations for an LC–MS–MS method have been identified. This includes (a) the obvious restriction of selectivity of the detection system when similar, but not identical compounds have to be analyzed (e.g., identical MRM ion pairs) and (b) the yield of selected ions depends on co-eluting matrix compounds resulting from unselective and/or insufficient sample clean-up. Consequently the selective sample clean-up and/or the final chromatography prior to MS analysis may have to be improved. For complex biological and environmental sample matrices this may be unavoidable.

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