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Chemiluminescence detection of cannabinoids and related compounds with acidic potassium permanganate

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This is the first report of chemiluminescence from the reaction of cannabinoids with acidic potassium permanganate, which we have applied to the high performance liquid chromatography (HPLC) determination of cannabidiol (CBD) in industrial-grade hemp. The intensities of the light-producing reactions with two commercially available cannabinoid standards were compared to that of seven model phenolic analytes. Resorcinol, representing the parent phenolic moiety of the cannabinoid class, was shown to react with the permanganate reagents in a manner more similar to phenol than to its hydroxyphenol positional isomers, pyrocatechol and hydroquinone. Alkyl substituents on the phenolic ring, however, have a considerable impact on emission intensity that is dependent upon the position of the groups and the composition of the permanganate reagent. This analytical approach has potential for the determination of other cannabinoids including Δ^9-tetrahydrocannabinol in drug-grade cannabis. Copyright © 2012 John Wiley & Sons, Ltd.

Keywords: chemiluminescence; cannabinoids; cannabis; cannabidiol; cannabigerol; THC

Introduction

Chemiluminescence (the emission of light from an electronically excited product of a chemical reaction) has been exploited as a highly sensitive mode of detection for many pharmaceuticals and controlled drugs,[1–8] including opioids (e.g. morphine,[9] heroin[10]), tranquilizers (e.g. loprazolam,[11] nitrazepam[12]) stimulants (e.g. methamphetamines,[13] cocaine[14]), and hallucinogens (e.g. psilocin,[15] phencyclidine[16]). In contrast, there has been very little study of chemiluminescence reactions with cannabinoids (the terpenophenolic secondary plant metabolites largely responsible for the bioactivity of cannabis[17]). Over 90 different cannabinoids have been identified,[17] with the five major compounds reported to be cannabinol (CBN), Δ^9-tetrahydrocannabinol (THC), cannabidiol (CBD), cannabigerol (CBG) and cannabichromene (CBC) (Figure 1).[18] The first isolated was CBN,[19] a degradation product of the psychoactive THC present in drug-grade cannabis.[20] CBD is the most concentrated cannabinoid in non-drug varieties.[21]

In 1972, Monforte *et al.*[22] published a brief note of their observation that ethanolic extracts of hashish and solutions of its major constituents (CBN, CBD, and THC) inhibited the haemoglobin-catalyzed chemiluminescence reaction of luminol and hydrogen peroxide, but this approach has not been further explored. Over a decade later, Abbott[23] investigated the chemiluminescence reactions of acidic potassium permanganate with illicit drugs and related molecules, including CBD. Subsequent studies showed that permanganate reagents generate an intense emission (emanating from an electronically excited Mn(II) species[24]) with many phenolic compounds,[4] but Abbott did not observe a significant response for CBD, in comparison with the large signal obtained for the phenolic opioid morphine.[7,23]

We recently gained insight into the vast differences in intensity elicited by different phenols and related compounds with this reagent by comparing the responses for nine hydroxyl/amino disubstituted benzene positional isomers.[25] In each case, the light-producing pathway of the *meta*-substituted compound was found to be far slower (resulting in much lower chemiluminescence intensities in flow analysis systems) than that of the *ortho* or *para* isomers. Furthermore, we have shown that an initial partial reduction of permanganate reagents can be used to generate relatively high concentrations of the key Mn(III) precursor to the emitter (Figure 2),[26,27] resulting in considerable increases in reaction rates and chemiluminescence intensities. This effect is most prominent for otherwise slow-reacting analytes, such as phenols without additional hydroxyl, alkoxyl or amino substituents (e.g. synephrine and tyrosine), or phenols with those substituents in the *meta* position (e.g. resorcinol and fenoterol).[25–27]

Considering that cannabinoids contain *meta*-substituted dihydroxybenzene groups or cyclic alkoxyl analogues (Figure 1), an examination of the chemiluminescence reactions of these and related compounds with permanganate offers not only a novel approach for their detection, but also a greater understanding of the relationship between analyte structure and chemiluminescence intensity with this widely utilized reagent.

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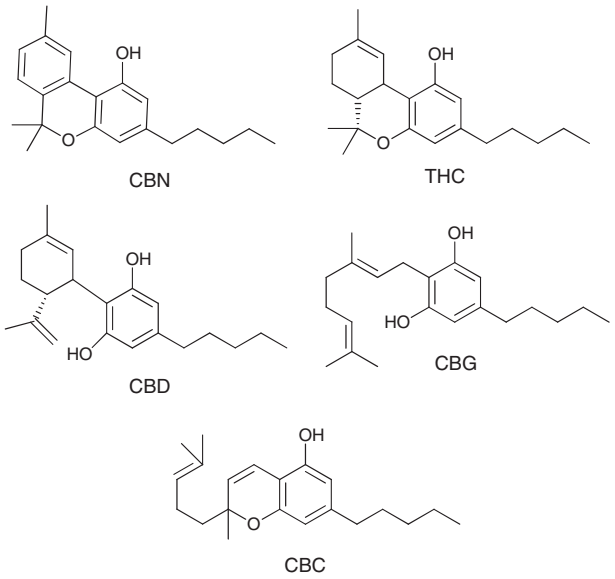

Figure 1. Chemical structures of the major cannabinoids: cannabinol (CBN), Δ^9-tetrahydrocannabinol (THC), cannabidiol (CBD), cannabigerol (CBG) and cannabichromene (CBC).

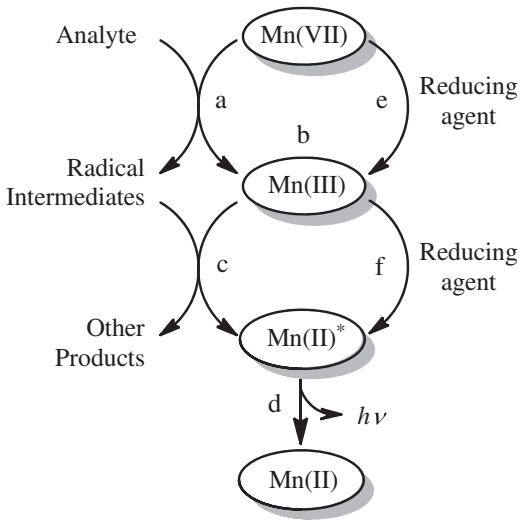

Figure 2. Generalized light-producing pathway of permanganate chemiluminescence: (a) multistep reduction of permanganate with analyte-dependent rate of reaction; (b) Mn(III) intermediate stabilized by complexing agents such as polyphosphate[28] or under highly acidic conditions; (c) radicals derived from the analyte react with Mn(III) to form the $Mn(II)^*$ emitter;[28] (d) radiative decay to the ground state product;[24] (e) reaction of permanganate with reducing agents, either during preparation (e.g. thiosulfate), or merged with the reagent on-line (e.g. formic acid or formaldehyde) to form a greater pool of Mn(III), which can enhance the chemiluminescence signal from the analyte;[25,27] and (f) unwanted reaction of reducing agent with Mn(III). Reprinted from Analytica Chimica Acta, 707, T. Slezak, Z.M. Smith, J.L. Adcock, C.M. Hindson, N.W. Barnett, P.N. Nesterenko, P.S. Francis, Kinetics and selectivity of permanganate chemiluminescence: A study of hydroxyl and amino disubstituted benzene positional isomers, 121–127. (©2011, with permission from Elsevier.)

Herein we examine the chemiluminescence response of CBD and CBG in comparison with seven model phenolic compounds, and demonstrate the analytical potential of these reactions through the determination of CBD in an industrial hemp leaf sample.

Experimental

Flow injection analysis (FIA)

A simple two-line FIA manifold was constructed as previously described.[29] The analytes were injected (70 μl) into a carrier stream, which merged at a T-piece with the acidic potassium permanganate shortly prior to entering the coil-tubing detection flow-cell. Both lines were pumped at a flow rate of 3.5 ml min^{-1}. The flow-cell was mounted flush against the window of a photomultiplier tube (Electron Tubes model 9828SB; ETP, NSW, Australia) encased in a light-tight housing and powered by a stable power supply at 950 V. Chemiluminescence intensities were documented with a chart recorder and measured manually using peak height. Details of the stopped-flow experiments are included as electronic supporting information.

High performance liquid chromatography (HPLC)

Chromatographic analysis was carried out using an Agilent Technologies 1200 Series liquid chromatography system, equipped with a solvent degasser, quaternary pump and autosampler (Agilent Technologies, Vic, Australia). Agilent Chemstation software was used for system control and data acquisition. Chemiluminescence detection was employed using the manifold described above, by replacing the carrier stream (and injection valve) with the exit line from the column. The chemiluminescence reagent was propelled at 1.2 ml min^{-1} using a Gilson Minipuls 3 peristaltic pump (John Morris Scientific, NSW, Australia). An analogue-to-digital interface box (Agilent) was used to convert detector signals. Extract solutions were filtered through a 0.45 μm nylon membrane prior to HPLC analysis. All separations were carried out using an Agilent Eclipse XDB-C_{18} column (5 μm; 4.6 mm × 150 mm) with an injection volume of 10 μl and mobile phase flow rate of 0.5 ml min^{-1}. The mobile phase was initially 100% water and increased linearly to 100% methanol over 30 min. 100% methanol was held for a further 30 min before the original conditions were restored and held for 5 min. Quantitative results were obtained using peak areas. Details of the liquid chromatography-mass spectrometry are included as electronic supporting information.

Extraction from hemp

Samples were prepared by solvent extraction from hemp leaf powder prepared as follows: industrial-grade hemp leaf (Commins Stainless Manufacturing Farm, Whitton, NSW, Australia) was collected and stored at room temperature, washed under water and dried at 60 °C, and then ground into a powder and passed through a 368 μm screen. Cannabinoids were extracted from 5 g of the powder with 300 ml of solvent (ethyl acetate, hexane, chloroform, ethanol or petroleum ether) for 1.5 h at boiling temperature using a Soxhlet apparatus. Another 300 ml of solvent was added and the extraction repeated for another hour. The two extracts were combined and then evaporated to dryness under vacuum at 40 °C, and stored at 4 °C until use.

Reagents

Standard solutions of CBD and CBG were sourced from Sapphire Bioscience (NSW, Australia). Potassium permanganate and ethanol were obtained from Chem Supply (SA, Australia). Sodium thiosulfate (anhydrous), sodium polyphosphate (crystals, +80 mesh, 96%), pyrocatechol, phenol, *t*-butylcatechol, resorcinol,

hydroquinone, orcinol and olivetol were supplied by Sigma-Aldrich (NSW, Australia). Methanol, petroleum ether (40–60 °C) and sulfuric acid (98%) were supplied by Merck (Vic, Australia). HPLC-grade methanol and ammonium formate were supplied by Ajax (NSW, Australia).

The standard permanganate reagent was prepared daily by dissolution of sodium polyphosphate (10 g L^{-1}) in water, adding potassium permanganate (1.0 mM) and adjusting to pH 2.5 using sulfuric acid. The enhanced permanganate reagents were prepared by dissolution of sodium polyphosphate (10 g L^{-1}) in water, adding potassium permanganate (1.9 mM), adjusting to pH 2.5 and then adding sodium thiosulfate (0.6 or 1.0 mM), using a small volume of a 0.1 M solution.

Results and discussion

Our initial investigations into the use of permanganate chemiluminescence for this application were conducted with seven readily available model analytes (Figure 3). Three dihydroxybenzene isomers – resorcinol, hydroquinone, and pyrocatechol – were included for direct comparison to our previous study,[25] and because resorcinol represents the parent phenolic moiety of the cannabinoid class. Phenol was included to test our previous postulation that the light producing pathways of *meta*-substituted dihydroxybenzene derivatives (e.g. resorcinol,[25] fenoterol,[27] and by extension, cannabinoids) with this reagent are similar to that of phenols without hydroxyl/amino groups. We selected orcinol, olivetol and *t*-butylcatechol to gain insight into the effects of the alkyl substituents on the phenolic ring of cannabinoid compounds. In initial experiments, stock analyte solutions (1×10^{-3} M) were prepared in methanol and diluted to 1×10^{-5} M in water.

The chemiluminescence intensities for each analyte upon reaction with the standard reagent and two 'enhanced' permanganate reagents containing relatively high concentrations of Mn(III) (optimized for related phenolic compounds in our previous study[27]) were established using FIA methodology (Figure 4). Responses for resorcinol were similar to phenol, where both exhibited very low signals with the standard reagent (white columns), but far greater enhancement than pyrocatechol and hydroquinone when the reagents containing Mn(III) were used (grey and black columns). An examination of the chemiluminescence intensity over time using stopped-flow instrumentation (Figures S1a and S1b; electronic supporting information) confirmed that in both cases the enhancement was largely a consequence of a significant increase in the rate of the otherwise slow light-producing reaction

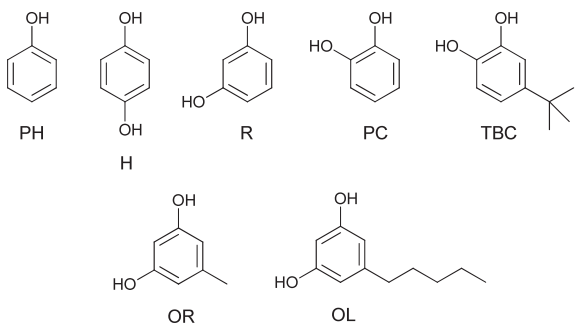

Figure 3. Structures of the seven model compounds: phenol (PH), hydroquinone (H), resorcinol (R), pyrocatechol (PC), *t*-butylcatechol (TBC), orcinol (OR) and olivetol (OL).

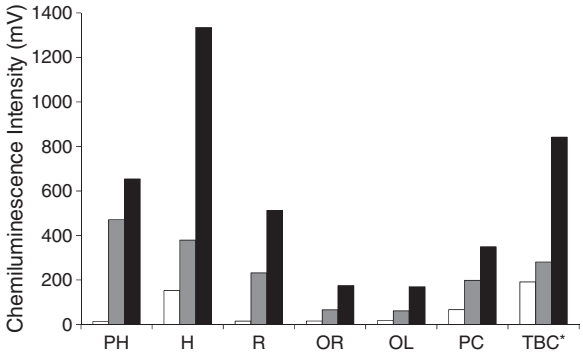

Figure 4. Relative chemiluminescence intensities for model compounds: phenol (PH), hydroquinone (H), resorcinol (R), orcinol (OC), olivetol (OL), pyrocatechol (PC) and *t*-butylcatechol (TBC) with the standard permanganate reagent (white columns) and two enhanced permanganate reagents (grey and black columns), using FIA methodology. The relative standard deviation for replicate injections was < 2%. *The analytes were prepared at a concentration of 1×10^{-5} M, except for TBC which was at 1×10^{-6} M. The aqueous carrier and analyte solutions each contained 1% methanol. The enhanced permanganate reagents were prepared by adding an equivalent concentration of 0.6 mM (grey columns) or 1 mM (black columns) sodium thiosulfate to a 1.9 mM permanganate reagent.

with the standard reagent. The use of the enhanced reagent improved the limits of detection for phenol and resorcinol from 1×10^{-6} M to 8×10^{-9} M, which is superior to most values reported for these compounds with permanganate chemiluminescence,[30–35] and equivalent to those of a procedure incorporating pre-concentration of the analyte within a solid-phase reactor prior to chemiluminescence detection.[36]

The responses for *t*-butylcatechol showed a similar trend to that of pyrocatechol and hydroquinone, where the relatively large signals with the standard reagent were only enhanced by 4- to 9-fold by increasing the concentration of Mn(III) in the reagent. The absolute intensities, however, were much higher for *t*-butylcatechol than the other analytes (NB: the data for *t*-butylcatechol in Figure 4 was obtained using an order of magnitude lower concentration). In contrast, the presence of alkyl groups at the C5 position of orcinol and olivetol reduced the chemiluminescence signal with the enhanced permanganate reagents (compared to resorcinol).

The cannabinoid standards used in this study (cannabidiol (CBD) and cannabigerol (CBG), Figure 1) were less soluble in aqueous solution than the model compounds, and therefore subsequent comparisons were conducted with the analyte stocks (1×10^{-3} M) prepared in methanol and then diluted with 1:1 methanol:water. A carrier stream of 1:1 methanol:water was also employed. Under these conditions, the same general trends were observed for the chemiluminescence responses, but the absolute signals were larger (Figure 5). Although the alkyl groups at the C5 position in orcinol and olivetol caused a reduction in the chemiluminescence response with the enhanced permanganate reagents (compared to resorcinol), the additional alkyl moieties at the C2 position in CBD and CBG imparted a large (~50-fold) increase in intensity with the standard permanganate reagent, similar to the effect of the alkyl group in *t*-butylcatechol (Figures S1 and S2) and partially ameliorated the effect of the pentyl group on the response with the enhanced reagent.

Under the separation conditions described in the experimental section, the elution time for CBD was 32.8 min and the limit of detection was 1×10^{-6} M. The CBG standard was found to co-elute with CBD, but a preliminary examination of the hemp-leaf extracts using mass spectroscopy revealed the absence of any

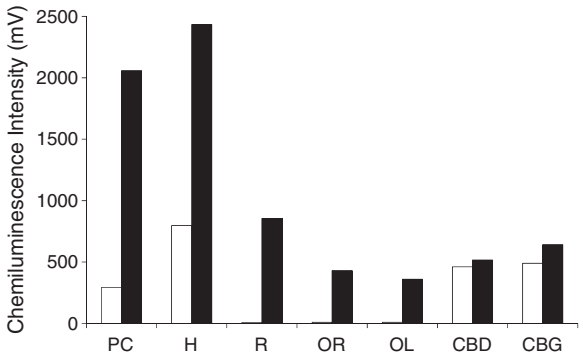

Figure 5. Relative chemiluminescence intensities for pyrocatechol (PC), hydroquinone (H), resorcinol (R), orcinol (OC), olivetol (OL), cannabidiol (CBD) and cannabigerol (CBG) with the standard permanganate reagent (white columns) and the enhanced permanganate reagent (black columns), using flow injection analysis methodology. The relative standard deviation for replicate injections was < 2%. All analytes were prepared at a concentration of 1×10^{-5} M in 1:1 water:methanol. The enhanced permanganate reagent was prepared by adding an equivalent concentration of 1 mM sodium thiosulfate to a 1.9 mM permanganate reagent.

significant concentration of CBG in the sample. Ethyl acetate and ethanol were found to extract similar quantities of CBD from the sample, which were higher than the concentrations extracted by the other three solvents. Figure 6 shows the chromatograms obtained for the ethyl acetate extract of a male hemp leaf sample, comparing the standard and enhanced permanganate reagents. In agreement with the FIA results, greater signal intensities for CBD were obtained with the permanganate reagents containing high initial concentrations of Mn(III). Numerous other peaks with retention times below 30 min show some degree of enhancement, suggesting that the reagent will also improve the detection of related analytes within cannabis samples.

To determine the concentration of CBD in the hemp leaf extracts, a linear calibration was prepared using 10 standard solutions between 5×10^{-5} M and 5×10^{-4} M ($R^2 = 0.9999$) (Figure S3). The concentration found in male and female hemp leaf extracts was 2.0×10^{-4} M and 2.2×10^{-4} M, which equated to 0.75% and 0.83% CBD (by mass) in the original dried leaf samples. While it should be noted that the quantification of neutral cannabinoids is complicated by the degree that their acidic precursors are converted to the target analytes during extraction, these values are well within the range reported by de Meijer and co-workers (0.55% to 1.8%) for dried leaf powders from 62 non-drug phenotype cannabis samples from a diverse range of sources.[37]

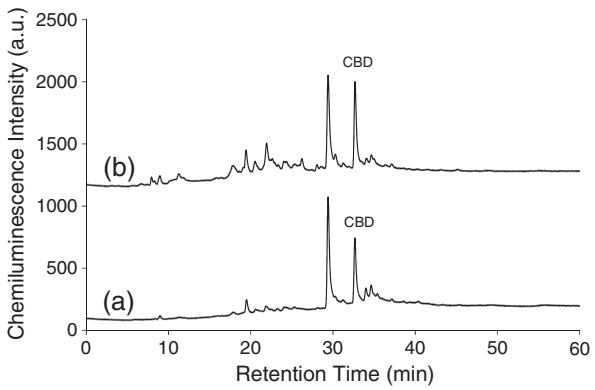

Figure 6. Chromatograms for the separation of an ethyl acetate extract from a male hemp leaf sample, with chemiluminescence detection using: (a) standard permanganate reagent; and (b) enhanced permanganate reagent prepared by adding an equivalent concentration of 1.0 mM sodium thiosulfate to a 1.9 mM permanganate reagent containing 1% sodium polyphosphate and adjusted to pH 2.5.

Conclusion

The *meta*-substituted dihydroxybenzene, resorcinol, was shown to react with the permanganate reagents in a more similar manner to unsubstituted phenol, rather than pyrocatechol or hydroquinone, for which simple oxidation pathways to benzoquinone products are available. The alkyl groups at the C2 and C5 positions of the cannabinoid benzene ring have very different effects on chemiluminescence intensity with acidic potassium permanganate. The pentyl group at C5 reduced the response with the enhanced reagent, whereas the larger alkyl moiety at C2 increased the intensity by approximately 50-fold with the standard reagent and 1.4- to 1.6-fold with the enhanced reagent (compared to olivetol), which we attribute to the relative stability of the respective radical oxidation products (Figure 2). The chemiluminescence reaction of cannabinoids with acidic potassium permanganate was successfully applied to the HPLC determination of CBD in industrial-grade hemp, which gave a result consistent with previous findings. This analytical approach could be extended to other cannabinoids, such as THC in drug-grade cannabis.

References

[1] M. Tsunoda, K. Imai. Analytical applications of peroxyoxalate chemiluminescence. *Anal. Chim. Acta* **2005**, *541*, 13.
[2] B. A. Gorman, P. S. Francis, N. W. Barnett. Tris(2,2′-bipyridyl)ruthenium (II) chemiluminescence. *Analyst* **2006**, *131*, 616.
[3] C. A. Marquette, L. J. Blum. Applications of the luminol chemiluminescent reaction in analytical chemistry. *Anal. Bioanal. Chem.* **2006**, *385*, 546.
[4] J. L. Adcock, P. S. Francis, N. W. Barnett. Acidic potassium permanganate as a chemiluminescence reagent—A review. *Anal. Chim. Acta* **2007**, *601*, 36.
[5] A. J. Brown, P. S. Francis, J. L. Adcock, K. F. Lim, N. W. Barnett. Manganese(III) and manganese(IV) as chemiluminescence reagents: A review. *Anal. Chim. Acta* **2008**, *624*, 175.
[6] P. S. Francis, J. L. Adcock, J. W. Costin, S. D. Purcell, F. M. Pfeffer, N. W. Barnett. Chemiluminescence detection of opium poppy (*Papaver somniferum*) alkaloids. *J. Pharm. Biomed. Anal.* **2008**, *48*, 508.
[7] J. L. Adcock, C. J. Barrow, N. W. Barnett, X. A. Conlan, C. F. Hogan, P. S. Francis. Chemiluminescence and electrochemiluminescence detection of controlled drugs. *Drug Test. Analysis* **2011**, *3*, 145.
[8] A. M. García-Campaña, L. Gámiz-Gracia, J. F. Huertas-Pérez, F. J. Lara, in *Chemiluminescence and Bioluminescence: Past, Present and Future*, (Ed: A. Roda). RSC, Cambridge, UK, **2011**, pp. 227–288.
[9] R. W. Abbott, A. Townshend, R. Gill. Determination of morphine by flow injection analysis with chemiluminescence detection. *Analyst* **1986**, *111*, 635.
[10] K. M. Agg, A. F. Craddock, R. Bos, P. S. Francis, S. W. Lewis, N. W. Barnett. A Rapid Test for Heroin (3,6-Diacetylmorphine) Based on Two Chemiluminescence Reactions. *J. Forensic Sci.* **2006**, *51*, 1080.
[11] A. R. J. Andrews, A. Townshend. Chemiluminescence detection of the benzodiazepine loprazolam. *Anal. Chim. Acta* **1989**, *227*, 65.
[12] V. David, R. M. Marín Saèz, J. V. García Mateo, J. Martínez Calatayud. Enhanced chemiluminescent determination of chloramphenicol and related nitro compounds by 'on-line' photochemical reaction. *Analyst* **2000**, *125*, 1313.
[13] S. Nakamura, M. Wada, B. L. Crabtree, P. M. Reeves, J. H. Montgomery, H. J. Byrd, *et al.* A sensitive semi-micro column HPLC method with peroxyoxalate chemiluminescence detection and column switching for determination of MDMA-related compounds in hair. *Anal. Bioanal. Chem.* **2007**, *387*, 1983.

[14] Q. Song, G. M. Greenway, T. McCreedy. Tris(2,2′-bipyridine)ruthenium(II) electrogenerated chemiluminescence of alkaloid type drugs with solid phase extraction sample preparation. *Analyst* **2001**, *126*, 37.
[15] N. Anastos, S. W. Lewis, N. W. Barnett, D. N. Sims. The Determination of Psilocin and Psilocybin in Hallucinogenic Mushrooms by HPLC Utilizing a Dual Reagent Acidic Potassium Permanganate and Tris (2,2′-bipyridyl)ruthenium(II) Chemiluminescence Detection System. *J. Forensic Sci.* **2006**, *51*, 45.
[16] R. Chiba, M. Fukushi, A. Tanaka. Simultaneous Determination of Phencyclidine and Its Metabolites in Rat Urine by High-Performance Liquid Chromatography. *Anal. Sci.* **1998**, *14*, 979.
[17] J. T. Fischedick, A. Hazekamp, T. Erkelens, Y. H. Choi, R. Verpoorte. Metabolic fingerprinting of *Cannabis sativa* L., cannabinoids and terpenoids for chemotaxonomic and drug standardization purposes. *Phytochemistry* **2010**, *71*, 2058.
[18] G. Appendino, S. Gibbons, A. Giana, A. Pagani, G. Grassi, M. Stavri, *et al*. Antibacterial Cannabinoids from *Cannabis sativa*: A Structure-Activity Study. *J. Nat. Prod.* **2008**, *71*, 1427.
[19] T. B. Wood, W. T. N. Spivey, T. H. Easterfield. III.—Cannabinol. Part I. *J. Chem. Soc. Trans.* **1899**, *75*, 20.
[20] J. Levine. Origin of Cannabinol. *J. Am. Chem. Soc.* **1944**, *66*, 1868.
[21] N. J. Doorenbos, P. S. Fetterman, M. W. Quimby, C. E. Turner. Cultivation, extraction, and analysis of *Cannabis Sativa* L. *Ann. N. Y. Acad. Sci.* **1971**, *191*, 3.
[22] J. Monforte, K. Weber, L. Grlic. Effect of major cannabinoids on the chemiluminescence of luminol. *Acta Pharm. Jugoslav*. **1972**, *22*, 27.
[23] R. W. Abbott, Ph.D. thesis, University of Hull, UK, **1987**.
[24] J. L. Adcock, P. S. Francis, T. A. Smith, N. W. Barnett. The characteristic red chemiluminescence from reactions with acidic potassium permanganate: further spectroscopic evidence for a manganese (II) emitter. *Analyst* **2008**, *133*, 49.
[25] T. Slezak, Z. M. Smith, J. L. Adcock, C. M. Hindson, N. W. Barnett, P. N. Nesterenko, *et al*. Kinetics and selectivity of permanganate chemiluminescence: A study of hydroxyl and amino disubstituted benzene positional isomers. *Anal. Chim. Acta* **2011**, *707*, 121.
[26] T. Slezak, J. M. Terry, P. S. Francis, C. M. Hindson, D. C. Olson, D. K. Wolcott, *et al*. Autocatalytic Nature of Permanganate Oxidations Exploited for Highly Sensitive Chemiluminescence Detection. *Anal. Chem*. **2010**, *82*, 2580.
[27] P. S. Francis, C. M. Hindson, J. M. Terry, Z. M. Smith, T. Slezak, J. L. Adcock, *et al*. Enhanced permanganate chemiluminescence. *Analyst* **2011**, *136*, 64.
[28] C. M. Hindson, P. S. Francis, G. R. Hanson, J. L. Adcock, N. W. Barnett. Mechanism of Permanganate Chemiluminescence. *Anal. Chem.* **2010**, *82*, 4174.
[29] G. P. McDermott, P. S. Francis, K. J. Holt, K. L. Scott, S. D. Martin, N. Stupka, *et al*. Determination of intracellular glutathione and glutathione disulfide using high performance liquid chromatography with acidic potassium permanganate chemiluminescence detection. *Analyst* **2011**, *136*, 2578.
[30] W. Cao, X. Mu, J. Yang, W. Shi, Y. Zheng. Flow injection-chemiluminescence determination of phenol using potassium permanganate and formaldehyde system. *Spectrochim. Acta A* **2007**, *66A*, 58.
[31] L. Li, X. Chen, H. Sun. *Shanghai Huanjing Kexue* **2005**, *24*, 266.
[32] N. T. Deftereos, A. C. Calokerinos, C. E. Efstathiou. Flow injection chemiluminometric determination of epinephrine, norepinephrine, dopamine and L-dopa. *Analyst* **1993**, *118*, 627.
[33] B. Gómez-Taylor Corominas, M. Catalá Icardo, L. Lahuerta Zamora, J. V. García Mateo, J. Martínez Calatayud. A tandem-flow assembly for the chemiluminometric determination of hydroquinone. *Talanta* **2004**, *64*, 618.
[34] S.-L. Fan, L.-K. Zhang, J.-M. Lin. Post-column detection of benzenediols and 1,2,4-benzenetriol based on acidic potassium permanganate chemiluminescence. *Talanta* **2006**, *68*, 646.
[35] J. Dolejšová, M. Polášek, P. Solich, R. Karlícek. Determination of Dihydroxyphenols by Flow Injection Analysis (fia) with Chemiluminescence Detection. *Folia Pharm. Univ. Carol.* **2005**, *33*, 67.
[36] B. Gómez-Taylor Corominas, J. V. García Mateo, L. Lahuerta Zamora, J. Martínez Calatayud. Determination of Phenol by Preconcentration-Direct Chemiluminescence in a FIA Assembly. *Anal. Lett.* **2005**, *38*, 499.
[37] E. P. M. de Meijer, H. J. Kamp, F. A. Eeuwijk. Determination of Phenol by Preconcentration-Direct Chemiluminescence in a FIA Assembly. *Euphytica* **1992**, *62*, 187.

