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Parallel microscope-based fluorescence, absorbance and time-of-flight mass spectrometry detection for high performance liquid chromatography and determination of glucosamine in urine

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ABSTRACT

A parallel microscope-based laser-induced fluorescence (LIF), ultraviolet–visible absorbance (UV) and time-of-flight mass spectrometry (TOF-MS) detection for high performance liquid chromatography (HPLC) was achieved and used to determine glucosamine in urines. First, a reliable and convenient LIF detection was developed based on an inverted microscope and corresponding modulations. Parallel HPLC-LIF/UV/TOF-MS detection was developed by the combination of preceding Microscope-based LIF detection and HPLC coupled with UV and TOF-MS. The proposed setup, due to its parallel scheme, was free of the influence from photo bleaching in LIF detection. Rhodamine B, glutamic acid and glucosamine have been determined to evaluate its performance. Moreover, the proposed strategy was used to determine the glucosamine in urines, and subsequent results suggested that glucosamine, which was widely used in the prevention of the bone arthritis, was metabolized to urines within 4 h. Furthermore, its concentration in urines decreased to 5.4 mM at 12 h. Efficient glucosamine detection was achieved based on a sensitive quantification (LIF), a universal detection (UV) and structural characterizations (TOF-MS). This application indicated that the proposed strategy was sensitive, universal and versatile, and it was capable of improved analysis, especially for analytes with low concentrations in complex samples, compared with conventional HPLC-UV/TOF-MS.

1. Introduction

High performance liquid chromatography (HPLC) has been widely used in a conjunction with ultraviolet-visible absorbance (UV) detection and time-of-flight mass spectrometry (TOF-MS) to seek for comprehensive determinations [1,2]. Its most conventional configuration was tandem HPLC-UV/TOF-MS [3,4]. However, the linear range of conventional TOF-MS was unsatisfactory in quantifications [5]. In addition, analytes with low concentration were usually accompanied by poor signal to noise ratio in a complex background [6,7]. Although UV detection has excellent quantitative capabilities, its achieved sensitivity was limited [8,9]. Overall, conventional HPLC-UV/TOF-MS had shown certain drawbacks in qualifications for low concentration analytes in complex samples.

Alternative optical devices have been coupled with MS to address the aforementioned problem [10–16]. Arriaga et al. reported HPLC-LIF/TOF-MS to determine doxorubicin and metabolites [17]. Moreover, Huhn et al. employed LIF/TOF-MS to determine labeled N-glycans [18]. Previous researches have highlighted excellent quantitative capabilities and high sensitivity of LIF [19–23]. Generally, the combination of LIF and TOF-MS has demonstrated significant potentials to address preceding quantitative issues.

High sensitivity in LIF was greatly depended on precise optical alignments, for which specific knowledge and professional experiences were vital in LIF detection [24–26]. A convenient strategy to achieve on-line LIF detection for HPLC would thus be of great values, particularly for its conjunction with TOF-MS. Moreover, the degradation products, which were from the photo bleaching in LIF determination, would be introduced to TOF-MS in a tandem scheme [27,28]. Therefore, a parallel LIF detection can ensure that analytes kept original status before their introductions to TOF-MS. However, LIF detection could only be applied for

Abbreviations: LIF, laser-induced fluorescence; UV, ultraviolet-visible absorbance; TOF-MS, time-of-flight mass spectrometry; HPLC, high performance liquid chromatography; DPSS, diode pump solid state; RhB, rhodamine B; RBITC, rhodamine B isothiocyanate; EIC, extracted ion chromatograph; TIC, total ion chromatograph

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fluorescent chemicals. Therefore, a convenient and reliable microscope-based LIF detection was thus designed to develop a parallel HPLC-LIF/UV/TOF-MS configuration. This was proposed as a sensitive, universal and versatile method.

Glucosamine has been widely used in the prevention of bone arthritis due to aging, because it can stimulate the metabolism of articular cartilages [29–32]. The chromatographic determination of glucosamine has been frequently coupled with pre-column derivatization [33–35]. Its quantification was reported by tandem triple quadrupole MS, and achieved sensitivities, however, were worse than its counterparts by LIF [36,37]. In this study, proposed HPLC-LIF/UV/TOF-MS was evaluated in the determination of glucosamine in urines.

In this work, an inverted microscope was used to develop a convenient and reliable microscope-based LIF detection, based on which a parallel HPLC-LIF/UV/TOF-MS was achieved. An analytical method was then established to achieve on-line separation, quantification and structural characterization for glucosamine in urines. This proposed strategy was of potentials to achieve improved analysis for analytes with low concentrations in complex samples.

2. Material and methods

2.1. Microscope-based LIF detection

The optical scheme of the proposed LIF microscope was indicated in Fig. 1(A). A 50 mW laser beam at 532 nm was produced by a diode pump solid state (DPSS) laser (Stone Laser, China), and it was modulated by a mechanical chopper (NDWSHB, China) at 524 Hz. By an aluminum reflector, the beam was introduced into an inverted microscope (COIC, China), and focused into a 100 μm I. D. capillary (Yongnian, China), which was adjusted to the beam waist. Fluorescence from the detection window was collected by the microscope, extracted by a high pass filter at 560 nm accompanied with a pinhole, and finally detected by photomultiplier tube (Hamamatsu, China). Meanwhile, a CMOS camera (Yigongfang, China) was used to record the vision under the microscope.

The modulation of LIF signal was presented in Fig. 1(B). The initialized signal from PMT was amplified and conditioned by a current-to-voltage preamplifier (Zolix Instruments, China). Then, the raw signal and the chopping frequency from the mechanic chopper were synchronously collected by a lock-in amplifier (Signal recovery, USA) as the detection signal and the reference

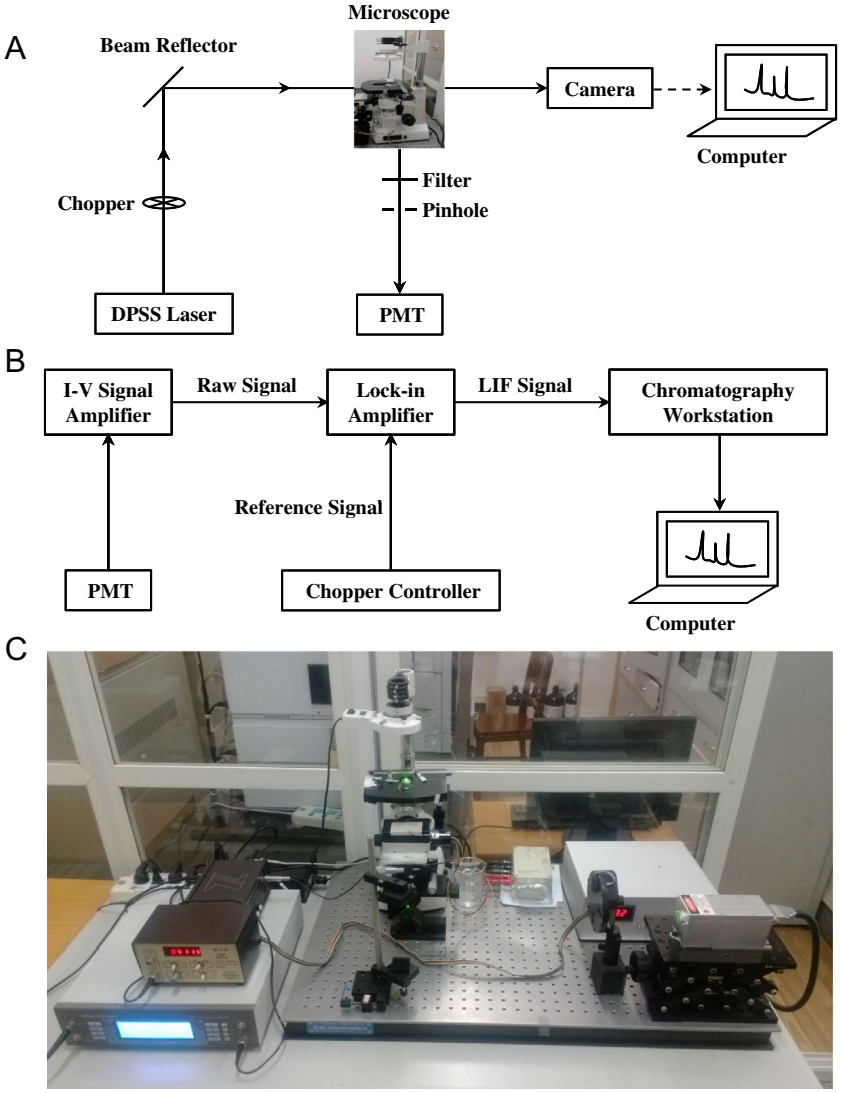

Fig. 1. The optical diagram and the signal modulation of the microscope-based LIF detection. (A: the optical diagram of the proposed LIF microscope; B: the modulation of LIF signal; C: the practical setup of the LIF inverted microscope.).